ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
Fiscal Year 1980

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

United States NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

Fiscal Year 1980

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National Institute of Child Health and Human Development

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NICHD ANNUAL REPORT

October 1, 1979, through September 30, 1980

OFFICE OF THE DIRECTOR

The National Institute of Child Health and Human Development conducts and supports research that focuses on the continuum of human development-from infancy and childhood, through adolescence and adulthood. The Institute has two major extramural research programs, the Center for Population Research (CPR) and the Center for Research for Mothers and Children (CRMC), an Epidemiology and Biometry Research Program which provides specialized collaboration with Institute staff and others as well as sponsorship and/or conduct of basic research in epidemiology and biostatistics, and an intramural research program where a variety of laboratory and clinical research is performed. Its project descriptions provide the bulk and essence of this annual report.

The focus of the Institute in all areas of its activities is on the special health problems of women, children and families and the means to prevent childhood conditions that result in disease, disability, and the major killers and cripplers of adult life. Within the current organizational framework, the Institute has developed a research strategy directed toward addressing the diversity of program interests and research problems which could be included within an extensive research mission. This strategy is based on a multidisciplinary mix of studies in reproductive and developmental biology, demography and epidemiology, and the behavioral and social sciences. These disciplines are arrayed across ten research program areas including; Pregnancy, Birth and The Infant; Sudden Infant Death Syndrome; Congenital Defects; Nutrition; Child and Adolescent Development; Mental Retardation; Fertility - Infertility; Contraceptive Development; Contraceptive Evaluation; and Population Dynamics.

Within the Center for Population Research the research fields addressed include reproductive biology and endocrinology, fertility-infertility, social and behavioral aspects of reproduction and population change, and contraceptive development and evaluation. Approximately forty per cent of the Institute's budget is invested in this area, which features a large contracts program. Much of this annual report includes summaries of individual contract projects supported within the CPR. The programs supported by the Center for Research for Mothers and Children direct multidisciplinary investigations on aspects of pregnancy and infancy, the Sudden Infant Death Syndrome, developmental biology and nutrition, mental retardation, and child and adolescent development.

It is the aim of the Institute to deal with health problems affecting families, both as a composite entity, and as individuals who form families. It approaches this task by studying problems affecting individuals prior to birth, at birth, and in childhood developmental activities both biological and behavioral. It is evident that successful research efforts instituted by NICHD can be particularly cost effective. Diseases, or disabilities, prevented before birth or in early childhood result in lifelong savings of adverse health costs to both the individual and society.

Two areas of study where this becomes evident are diabetes and mental retardation research. NICHD supported studies on high-risk pregnancy to enable diabetic mothers to have normal pregnancies and babies have the potential to prevent conditions that require long term care and treatment. Mental retardation research has the potential to prevent conditions that impair the quality of life and impart life long disability. The cost-benefits realized from research advances in these two areas alone are substantial.

The essential goal of the NICHD is to implement the one major national effort of multidisciplinary research to provide all couples with safe, effective, and inexpensive means to regulate fertility and to assure that all infants from conception and birth are provided opportunity for a healthful and productive adulthood. The content of this annual report presents significant achievements in all areas directed toward these goals. The Institute intends to continue and strengthen this research effort to preserve and maintain this valuable science base.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Center for Population Research
Office of the Director

Introduction

The Institute's Center for Population Research (CPR) continued to fulfill its responsibility for the primary Federal effort in population research. Significant progress has resulted again this year from the Center's support of research in the population sciences through grants and contracts for:

Fundamental biomedical research in the reproductive sciences relevant to problems of human fertility and infertility.

The development of new and improved methods for fertility regulation.

The evaluation of the safety and effectiveness of contraceptive methods currently in use.

Social and behavioral sciences research on the reproductive motivation of individuals, and the causes and consequences of population change.

Research in the reproductive sciences on fertility and infertility is concerned with the reproductive processes involved in the fertility of men, women, and animals with reproductive processes similar to those in human beings. The purposes of this program are: (1) the advancement of fundamental knowledge required for the maintenance of the reproductive health of men and women; (2) the identification and development of new leads for less hazardous and more effective methods of fertility regulation for use by men and women; and (3) the alleviation of infertility.

Studies in fertility and infertility are subsumed under the four major disciplines which comprise the reproductive sciences. These disciplines are: (a) reproductive endocrinology; (b) reproductive biology; (c) reproductive medicine; and (d) reproductive chemistry. Reproductive endocrinology includes the secretion, action, and metabolism of the reproductive hormones and the endocrine glands which secrete them. Reproductive biology includes the reproductive processes and events such as oocyte maturation, sperm production, ovulation, sperm maturation, sperm capacitation, gamete transport, fertilization, early embryonic development, implantation, reproductive behavior, and nutrition in animals. Reproductive medicine includes human nutrition, human infertility, human reproductive diseases and disorders, andrology, and clinical studies of human reproduction. Reproductive chemistry includes synthesis of reproductive peptides and steroids, isolation and purification of substances involved in reproduction; and chemical modification of substances involved in reproduction for increased efficacy and diminution of undesirable side effects.

The contraceptive development program involves research that will lead to the development of new and improved methods of fertility regulation for both men and women that are safe, effective, inexpensive, reversible, and acceptable to various population groups. The objective of this research is to develop an

array of methods that can be used successfully by couples with differing requirements living under a variety of circumstances. At all stages of research the overall aspect of product and method safety is emphasized.

Research activities included in contraceptive development are: (1) synthesis and biological evaluation of promising new compounds that may affect reproductive processes in the male or female; (2) the development of technology for improved administration of contraceptive drugs; (3) the development of improved vaginal and uterine contraceptives based on chemical or physical methods; (4) clinical trials of sex steroids and peptides for suppression of sperm production and the consequent development of chemical contraceptives for men; (5) clinical and toxicological evaluation of long-acting progestin as a female contraceptive; (6) laboratory studies and clinical trials to develop and evaluate antifertility methods based on periodic abstinence; and (7) studies required to clarify mechanisms of action of specific contraceptive drugs.

The program of research on contraceptive evaluation deals with the safety of various contraceptive methods which are currently in use, particularly with respect to long-term safety. Efforts are directed toward the identification of health risks associated with various methods, delineation of particular circumstances which increase the risks of specific methods, and clarification of the mechanisms by which specific adverse effects may arise.

Research on contraceptive evaluation includes the following: (1) Studies of steroid contraceptives to determine their effects on cancer and other neoplasia, heart disease, hypertension, thromboembolic disease, metabolic, nutritional, and immunologic disorders. Also included are studies of the role of the immune system in mediating adverse effects of oral contraceptives, evaluation of individual variation in pharmacologic response to contraceptive steroids, and evaluation of sequelae, especially the risk of cancer, after exposure to diethylstilbestrol in utero or during pregnancy. (2) Studies of intrauterine devices to determine their effects on the occurrence of serious gynecologic and obstetric disorders such as pelvic inflammatory disease, vaginal hemorrhage, uterine perforation, ectopic pregnancy, fetal loss, abruptio placenta, placenta previa, and infertility. (3) Studies of the medical sequelae of male and female sterilization and research to clarify factors related to return of fertility following anatomic reversal of sterilization in men and in women. (4) Studies of the effects of induced abortion on subsequent reproductive function, such as the occurrence of spontaneous abortion, prematurity, infertility, etc. (5) Research on the use-effectiveness of inadequately evaluated methods, such as the diaphragm and condom. (6) Studies of the health effects of contraceptive use by teenagers.

Research in the social and behavioral sciences deals with the factors governing variations in the growth, distribution, and characteristics of people and the impact of population changes on the health and welfare of individuals, families, and society as a whole. This research includes two major categories. The first involves studies of the causes of population change, including factors affecting the three components of population change: fertility, mortality, and migration. Research has emphasized factors affecting fertility, since this has been the largest and most variable component of population change in the United States. Research on the causes of variations in fertility is concerned primarily with

social, economic, and psychological determinants. Also included are such topics as choice of methods of fertility control, attitudes towards such methods, the effectiveness with which methods are commonly used, the number of children wanted and expected, changes in family structure and function, the desired timing of childbearing, the incidence of unwanted childbearing, illegitimacy, premarital sexual behavior and the use of contraception among teenagers. Research on the causes of migration, deals with factors affecting trends in internal migration, such as the movements between metropolitan and non-metropolitan areas, and international migration, particularly the large movements of illegal immigrants into the United States. Research in mortality includes studies of mortality differences between various social and economic groups and of social and economic factors affecting trends in mortality.

The second second major category in the social and behavioral sciences concerns studies of the consequences of population change and includes research on the personal, familial, and social consequences of changes and differentials in the components of population change (fertility, mortality, and migration) and the resulting characteristics and distribution of the population. Emphasis has been placed on the consequences of childbearing patterns for individuals, families, and society; for example, studies of the effects of maternal age, birth spacing, and birth order on the psychosocial development of the child, the economic welfare of the parents, the participation of women in the labor force and the stability of the family unit. Special attention is given to the consequences of teenage childbearing for mother and child. Research on the consequences of migration include the social and economic impact of large movements of people into or out of specific areas. Studies of the consequences of mortality include the impact on the size of specific age groups and the effects on family composition. Research on the consequences of population change also include studies of the social, economic, and environmental impacts of varying rates of population growth.

Research Results

Recent findings and achievements in population research supported by the Institute include the following:

1. A breakthrough in research on the mechanisms of action of gonadotropins has been attained by investigators who have provided evidence of the "internalization" of these reproductive peptide hormones. The gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), control ovulation and hormone secretion by the ovary as well as sperm production and hormone secretion by the testis. The newly revealed process of gonadotropin internalization supercedes the long-held tenet that gonadotropins bind to target cell surfaces and do not enter the interior of such cells.

2. Advances have been made in the synthesis and use of analogs of Gonadotropin Releasing Hormone (GnRH), which have potential application concerning female fertility and infertility. GnRH is normally produced in the brain and stimulates the pituitary gland to secrete gonadotropins which, in turn, induce ovulation. Normally cycling women who received injections of 50 micrograms of a long-acting GnRH analog on each of two successive days during mid-luteal phase exhibited short luteal phases and premature menstruation, as a consequence of the analog

inhibiting the secretion of hormones by the ovary.

3. Research results have shown that it is now possible to reliably demonstrate GnRH receptors in the pituitary gland and thus, to delineate the role of membrane GnRH receptors in determining the subsequent gonadotropin response to the hypothalamic hormone. This has provided the first available system to accurately measure GnRH receptor affinity and number. The significant finding of specific GnRH receptors in the ovary and the testes allows further studies to elucidate whether GnRH or analog therapy has a direct effect on ovarian and testicular receptors and steroidogenesis.

4. A new model has been developed for studying the mechanism of action of FSH, i.e., the ability of FSH to stimulate incorporation of labeled glucosamine into mucopolysaccharides of proteoglycans secreted by ovarian granulosa cells. The stimulation of this biosynthesis appears to be specific for FSH and, therefore, offers an attractive possibility of being developed as a new bioassay for FSH. This methodology would be very useful for understanding the relative roles of the different gonadotropins in reproductive physiology.

5. At fertilization the spermatozoon binds to the ovum, and it was previously thought that this binding process involved membrane-bonded acrosome granules. Research has now shown that these granules contain a sperm-specific protein named bindin. This protein, for the first time, has been isolated, purified, and its molecular weight ascertained.

6. A new assay technique has been developed for monitoring the fertility level of the human male's semen. Men attending a fertility clinic were studied with regard to the physico-chemical state of their sperm quality. The dye fluorescence of individual sperm nuclei were measured in a cytofluorograf interfaced to a minicomputer, which provided an index of DNA denaturation called alpha-t. The study has shown that abnormal sperm cannot resist denaturation like normal sperm. Tests sampled from sub-fertile or questionably fertile animals and men have been found to have a higher alpha-t index measurable by the cytofluorograf machine.

7. Efforts to develop improved means of fertility regulation through the synthesis and biological testing of analogs of Luteinizing Hormone Releasing Hormone (LHRH), also known as GnRH, have made progress in the area of antagonists. The synthesis activities have resulted in the development of antagonists that block ovulation in the rat at the 10 microgram level, which represents a three-fold improvement in activity. Antagonists administered to male rats result in pronounced reduction in testes and accessory organ weights. Preliminary evidence that ovulation can be blocked in non-human primates has also been obtained.

8. The capability of synthesizing superactive agonists of LHRH and the knowledge of their paradoxical effects in both male and female laboratory animals has led to initiation of clinical trials in human volunteers. The purpose of these initial trials is to investigate the potential of these drugs as male contraceptives. In addition, several regimens of drug administration are being utilized and evaluated in order to focus on a pragmatic approach in later studies.

9. The synthetic chemical facility has been active in supplying important amino

acids to the LHRH program; synthesis of intermediates for prostaglandins and steroids; purification and analysis of gossypol, a potential male contraceptive; and synthesis of intermediates for the World Health Organization's program in human reproduction.

10. The biological drug testing facility has been significantly augmented through procurement of a large colony of cynomolgus monkeys, which is being used for assessment of the potential of LHRH analogs as male contraceptives and for studies on female contraceptive agents. The colony's availability will materially improve selection of feasible drugs and drug delivery systems for animal safety studies and clinical trials.

11. A study of myocardial infarction in young women on oral contraceptives has confirmed observations made in the United Kingdom. Current pill users were four times more likely to have a heart attack than nonusers. Women who both smoked heavily and were current pill users were found to be nearly 40 times as likely to have a heart attack as women who neither smoked nor used oral contraceptives. Past use of the pill, for more than 5 years, may also increase the risk of myocardial infarction.

12. A clinical trial on the effect of folic acid administration among women with cervical dysplasia and taking oral contraceptives suggests that folic acid may halt, and possibly reverse, cervical disease.

13. A study has confirmed that intrauterine device (IUD) use increases the risk of pelvic inflammatory disease, but also shows that oral contraceptive use and the use of barrier methods protect against upper genital tract infection. The study also confirms the finding that women with an IUD in place at the beginning of their second trimester of pregnancy are at increased risk for septic fetal loss. An IUD in place at conception, but removed in the first trimester of pregnancy, does not increase this risk.

14. Preliminary findings from ongoing studies suggest a higher rate of spontaneous abortion and fetal death among women with prior induced abortion. They also indicate that women with one previous induced abortion are not at higher risk of short gestation/low birth weight, complications of labor and other problems, when they carry their second pregnancy to term.

15. Higher relative income appears to increase completed fertility and to decrease female labor force participation. Also, higher relative income appears to induce both earlier childbearing and earlier female labor force participation. This study indicates, however, that female wage rates were the most important factor in recent fertility trends and trends in female labor supply. Increased female wage rates, mainly during the past 20 years, are associated with lower completed fertility, longer postponement of childbearing, and increased labor force participation.

16. Data from a longitudinal study point to seven aspects of couple interaction that are relevant to reproduction: communication, conflict, influence, decision making, bonding, division of labor, and adaptation to change. The first four directly affect the psychological sequences that lead to having a child. Marriage

and marital dissolution are affected by bonding. Division of labor is reflected in female role orientation and behavior.

17. The National Fertility Survey indicates that sterilization is becoming the major method of limiting family size in the United States. It is estimated that between two-thirds and three-fourths of once-married white couples will obtain sterilizing operations within 15 years after their last wanted birth. In a study of couples in the early years of marriage, 78 percent reported plans for sterilization after achieving their desired family size.

18. A longitudinal study of high school seniors found young women's orientation toward work was closely related to their marital and parenthood status. The importance of careers to married women decreased near the marriage date and decreased further when parenthood was imminent. Married men, compared with unmarried men, attached somewhat more importance to their careers, but parenthood had only a slight effect on men's orientation toward work. Among married women, mothers seemed slightly less satisfied with their career progress than did non-mothers, and dissatisfaction seemed linked to the timing of parenthood.

19. Studies addressing the factors associated with non-use of contraceptives have found increased but not extensive use of effective contraception by unmarried adolescent females. The major reason given for non-use of contraception was the belief that pregnancy could not occur, and another was that intercourse was unexpected. About 20 percent of the women in a national probability study had intercourse by age 16, and 66 percent had sexual relations by the time they were 18 years of age. Age at menarche was directly related to age at first intercourse and inversely related to the prevalence of premarital intercourse.

20. Studies of only children show no adverse effects of being an only child. Only children were found to be more like first-borns in larger families, and were shown to be slightly superior on cognitive abilities and achievement to children with siblings. On the whole, only children did not differ from children with siblings on a wide range of factors including physical development, utilization of medical care, behavioral and psychological factors, marital status, number of children they had, divorce rates, occupational choice, or levels of income.

Coordination and Communication

The activities of the Center to enhance the coordination of Federal population research programs and to foster the communication of biomedical and behavioral research information in the population sciences, are presented in this portion of the report since they are coordinated in the Office of the Director.

Interagency Committee on Population Research (ICPR), which is chaired by the Director of CPR, coordinates the population research activities supported by Federal agencies and facilitates the exchange of information and ideas among Federal programs involved with population research. The ICPR, established by the

Secretary of HEW on October 5, 1970, has been extended by the Secretary of HHS through June 30, 1982. It is comprised of representatives from the various Federal agencies concerned with research related to human population problems. The major products of the ICPR are the following two publications produced annually with the assistance of NICHD's Office of Planning and Evaluation.

Inventory and Analysis of Federal Population Research for fiscal year 1979 was issued by the ICPR. This report contains (1) a description of the background and role of the ICPR; (2) a statistical analysis with fiscal tables by research areas and supporting agencies; (3) a detailed inventory of all Federally supported population research projects classified according to the major research category; (4) a listing of projects started in FY 1979; and (5) a membership list of the ICPR.

Inventory of Private Agency Population Research for 1978 was published by the ICPR. This report provides information on the population research projects sponsored by the major U.S. private organizations in this field. The three principal private agencies involved with research in the population sciences, the Ford and Rockefeller Foundations and the Population Council, have provided the data on their research projects in the same way as the Federal agencies and the information has been published in the identical format as the Federal Inventory.

Population Research Monographs published by the Center through the Government Printing Office furthers the dissemination of information in the population sciences. These books provide a review of the state-of-the-art or report on progress in specialized areas of population research in the biomedical or social sciences, and indicate future research directions to obtain needed knowledge. The Center's conferences and workshops also continue to result in publications which constitute a valuable addition to the population research literature.

Social, Economic, and Health Aspects of Low Fertility, edited by Mr. Arthur Campbell, reports the proceedings of a conference sponsored jointly by the Center and the World Health Organization, which was held in 1977 in Washington, D.C. The book, published by G.P.O., includes the papers and discussions of the conference which describe recent fertility trends in countries with low fertility, hypotheses concerning the determinants and consequences of low levels of fertility, and future trends in fertility.

The Center enters progress and final reports of grants and contracts selected by our Branches into the National Technical Information Service (NTIS) in order to facilitate the dissemination of information on research findings and thus further the transfer of knowledge. NTIS, part of the U.S. Department of Commerce, is being utilized since they are the central source for the public sale of scientific and technical reports of U.S. Government sponsored research and development, and they also comprise the largest documentation center in the world for the public distribution of scientific publications. The following are the most recent reports entered by the Center into NTIS:

Accession No.	Title and Author(s)
PB 80-149859	The Social and Demographic Consequences of Teenage Childbearing for Urban Women by Harriet B. Presser
PB 80-184559	Employment of Women and Fertility by A.J. Jaffe and Jeanne Clare Ridley
PB 299518/AS	Population Manpower in the Social Sciences by J. Gregory Williams
PB 299425/AS	Urban Family Building Patterns: The Social Mobility-Fertility Hypothesis Reexamined by Basil G. Zimmer
PB 80-100621	REPSIM-B: A Computer Simulation Model of Human Reproduction (computer tape and report)
PB 80-100639	REPSIM-B (report only) by Jeanne Clare Ridley
PB 296977/AS	The Employed Woman: Family Planning and Career by Linda J. Beckman
PB 289051/AS	The Consequences of Age at First Childbirth: Educational Attainment by Kristin A. Moore, Linda J. Waite, Steven B. Caldwell, and Sandra L. Hofferth
PB 289052/AS	The Consequences of Age at First Childbirth: Family Size by Kristin A. Moore and Sandra L. Hofferth
PB 289053/AS	The Consequences of Age at First Childbirth: Marriage, Separation and Divorce by Kristin A. Moore, Linda J. Waite, Sandra L. Hofferth, and Steven B. Caldwell
PB 289054/AS	The Consequences of Age at First Childbirth: Labor Force Participation and Earnings by Sandra L. Hofferth, Kristin A. Moore, and Steven B. Caldwell
PB 289055/AS	The Consequences of Age at First Childbirth: Female Headed Families and Welfare Recipiency by Kristin A. Moore and Sandra L. Hofferth
PB 289056/AS	The Consequences of Age at First Childbirth: Causal Models by Sandra L. Hofferth and Kristin A. Moore

PB 289057/AS	The Consequences of Age at First Childbirth: Final Research Summary by Kristin A. Moore and Sandra L. Hofferth
PB 289058/AS	Consequences of Adolescent Childbearing for The Young Parent's Future Personal and Professional Life by Josefina J. Card
PB 289059/AS	Consequences of Adolescent Childbearing for the Mother's Occupational Attainment by Steven D. McLaughlin
PB 289050/AS	The Consequences of Age at First Childbirth: Complete set of 9 Volumes (listed above)
PB 288907/AS	Systems Approach to Vaginal Delivery of Contraceptive Drugs--Methodology and Mechanism for Absorption by William I. Higuchi
PB 292493/AS	Development of Orally-Active Dosage Forms for Steroids by J.P. Polidoro, D.L. Williams, K.R. St. John, and C.J. Hitchcock
PB 292378/AS	Detection of Ovulation in Chronically Instrumented Animals by Richard J. Blandau and Sheridan A. Halbert
PB 292414/AS	The Biology of the Oviduct and Gamete Transport by Richard J. Blandau
PB 292383/AS	Development of Bio-Erodable Contraceptive Implants by Gopi N. Gupta

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Center for Population Research
Contraceptive Development Branch

Fiscal year 1980 was marked by progress in the research and development program which has resulted in considerable expansion of the clinical trials component. Numerous approaches to fertility regulation which have been supported for a number of years at the laboratory level have now reached the stage where human clinical investigation of their potential for fertility regulation can be explored.

A. Product Development

Drug Development Program

The synthesis and biological testing of LHRH analogs has been primarily focused in the area of antagonists. It is important to recognize that prior to the discovery of paradoxical antifertility effects of LHRH agonists it was generally believed that the primary antifertility effects of this group of drugs will reside in the antagonists. During the past year the synthesis program has resulted in development of antagonists that block ovulation in the rat at the 10 μg level. This represents a three-fold improvement in activity. Preliminary evidence that ovulation can be blocked in non-human primates has also been obtained. Antagonists administered to male rats result in pronounced reduction in testes and accessory organ weights. Continued efforts in this area of research are likely to result in even more potent antagonists and to facilitate clinical exploration of these chemicals for fertility regulation in the U.S.A.

Activity in the prostaglandin (PG) area is centered on studies of the luteolytic potential of these drugs in non-human primates. Considerable effort is being expanded on development of new primate models for proper evaluation of the luteolytic potential of PGs.

One of the most difficult areas of synthesis has been the development of antiprogestational drugs. A major stumbling block has been the absence of viable leads. Preliminary biological testing suggests that a metabolite of the contraceptive progestin norethindrone, dihydro norethindrone, is a competitive inhibitor of progesterone and possesses antifertility activity. Medicinal chemists are trying to modify this molecule in order to improve its antifertility activity while at the same time to reduce its undesired other hormonal activities. Another potential lead is represented by D-seco steroids with weak progestational activity. It remains to be determined whether weak progestins based on the D-seco concept can serve as antiprogestins.

Synthetic Chemical Facility

Development of a successful synthesis program is frequently associated with the availability of key intermediates. During the past year the synthesis facility has been active in supplying, 1) important amino acids to the LHRH program, 2) synthesis of intermediates for PGs and steroids, 3) purification and

analysis of gossypol, a potential male contraceptive, 4) synthesis of intermediates for the WHO synthesis program.

Biological Drug Testing Facility

The testing program has been significantly augmented through procurement of a large colony of cynomolgus monkeys. This colony is being used for assessment of the potential of LHRH analogs as male contraceptives, as well as for studies on female contraceptive agents. Its availability will materially improve selection of candidate drugs and drug delivery systems for animal safety studies and clinical trials.

A substantial number of long-acting androgens and progestins continue to be evaluated for potency and longevity of action. Several compounds have been identified which exhibit sufficient activity to warrant extended assessment.

On an overall basis, the biological testing facility has fostered collaborative studies on drugs at both the national and international level.

Data generated at the facility are entered into computerized data analysis and storage systems are usually available to Contraceptive Development Branch staff through their in-house terminal within 24 hours of experiment termination.

Drug Delivery Systems and Oral Formulations

An implantable drug delivery based on caprolactone polymer is presently being evaluated in a 30 day Phase I clinical trial. Primary clinical evaluation of its safety, drug releasing properties and effects on endocrine parameters should facilitate considerations for longer-term studies.

Evaluation of the microcapsular injectable systems developed by a former contractor and by an outside source has been completed. The drug release profiles for our system was more of the zero-order release category than that of the competitors. Based on this information we intend to proceed with the fluidized bed prepared microcapsules containing levo-norgestrel. Studies will focus on optimization of the production of desired size microcapsules and thorough evaluation in several animal models.

Two bioerodable drug-delivery systems continue to be evaluated. Production of delivery system based on Chronomer™ has been optimized. Release profiles for norethindrone are very satisfactory. A major problem with the Chronomer™ system that needs to be resolved is the development of irritation at the implant site. Preliminary data suggest that the problem may be due to sterilization with gamma irradiation. Alternate sterilization techniques are being explored and we should be in position to make a decision on this specific system in about 6-8 months. The alternate bioerodable system is based on a different polymer. It appears to have some advantage over Chronomer™ in terms of manufacturing technology but has not been subjected to as an intensive biological evaluation. The irritation problem that has surfaced with Chronomer™ will be closely watched for with the alternative system.

Sustained-release oral formulations of currently available oral contraceptives

have been approved by the FDA for initial clinical studies. In animal experiments these preparations have reduced the peak blood absorption level and at the same time, they have extended the effective drug blood level. If a similar picture is obtained in human volunteers, it may result in improved safety of the drugs by reducing body burdens.

Device Development

In last year's report we stated that solicitation of proposals to develop improved barrier methods did not result in funded projects. During FY 1980 this trend has been reversed and several projects have been selected for funding. Their aim, together with ongoing studies, will be to develop improved materials for both diaphragms and cervical caps.

Clinical Studies (Female)

After a prolonged clinical hold, the FDA has indicated that efficacy trials of norethindrone enanthate can be initiated. As a condition, the Agency has stipulated that an additional two year rat toxicity study comparing norethindrone enanthate with a combination oral contraceptive be instituted. The Contraceptive Development Branch must still ascertain the design of this rather expensive safety study in view of the unprecedented nature of the FDA request. Data from WHO sponsored clinical trials indicate that this drug is efficacious when administered once every two months for a period of 6 months and with subsequent injections spaced at 3 month intervals.

During this fiscal year, we have also funded three clinical trials of different LHRH agonists. In two of the trials, the drugs are given by injection and in the third, the drug is administered as a nasal spray. These three clinical trials will delineate the potential of LHRH agonists as ovulation inhibitors and as luteolytic agents. They should also provide us with considerable basic information on the pharmacology of these drugs in normal women.

In the previous section (Device Development) we have indicated that increased research activity in barrier contraception is taking place. In the clinical area a similar surge of activity is evident.

Additional data have accumulated on the over-the-counter spermicidal foaming suppository called, Encare Oval™. There continues to be lower acceptability for Encare than for the diaphragm, although the pregnancy rates remain close at 12.7 per 1200 women - months of use for Encare and 9.1 for diaphragm users. The average number of months of use per Encare enrollee continues to be low. The product is acceptable for those who experience infrequent intercourse.

Initial studies of a polyurethane contraceptive vaginal sponge containing a spermicidal agent are being completed. The sponges cannot be left in the vagina for seven days as previously suggested. With shorter periods of time (2 days) the sponges were well tolerated. In post-coital tests reduced numbers of sperm were found in cervical mucus in all cases. We are currently negotiating for a multi-clinic Phase III clinical trial of the sponge in comparison with the diaphragm.

Because of increased interest in the cervical cap, the Contraceptive Development Branch is planning to initiate a large scale multi-clinic randomized study of the cap and the diaphragm. Conclusions of this study should provide sufficient efficacy data to permit the FDA to render a decision on marketing of the cap. At the present time, cervical caps are available only as experimental contraceptive devices. Recognizing that the available cervical caps need to be improved in order to be more acceptable, contracts are being negotiated for clinical evaluation of a custom-fitted cervical cap and the development of a cap insertion system together with a broader range of cap sizes.

Clinical Studies (Male)

The availability of superactive agonists of LHRH and the knowledge of their paradoxical effects in both male and female laboratory animals has led to initiation of clinical trials in human volunteers. The purpose of these initial trials will be to investigate the potential of these drugs as male contraceptives. Several regimens of drug administration will be evaluated in order to focus on a pragmatic approach in later studies. In this area of research we are benefiting from concurrent studies being carried out independently by investigators working in the area of infertility, as well as by those looking for a contraceptive approach.

Distribution Program

This program continues to be very viable. Numerous investigators working in the area of reproduction have benefited from the availability of high grade, uniform reagents. This program is also highly cost effective since many of the reagents can be procured in bulk quantities at a cost much lower than an individual investigator must pay for smaller quantities. No new reagents have been added to the Contraceptive Development Branch inventory.

B. Directed Fundamental Research

RIA Development

Interaction between NIH and WHO has resulted in the development of new reagents for the RIA of monkey LH. A kit incorporating an antiserum, monkey LH for radioiodination and a new LH reference preparation is now being evaluated by several laboratories. Once the testing period is completed, the kits will be made available to scientists through NIH and WHO distribution centers. As a result of this collaborative effort, we have also secured a new reference preparation for monkey FSH. The availability of these reagents will make a major contribution toward better understanding of primate reproduction.

Better understanding of testicular physiology is dependent on the ability to quantitate various testicular secretions. One of the intriguing proteins secreted by the testes is called the androgen binding protein or ABP. Quantitation of this protein under a variety of physiological and pharmacological conditions can foster development of new male contraceptives. Isolation of this protein in sufficient quantity to provide an immunogen for antibody production and for radioiodination is a primary mission of a continuing endeavor.

Follicular Inhibin

Follicular fluid contains a number of biologically active peptides. For some time we have been interested in a peptide that inhibits FSH serum levels. Injection of follicular fluid into monkeys at the time of menses appears to disrupt the cycle and produce a so-called insufficient luteal phase. Corpora lutea from animals injected with the fluid produce only limited amounts of progesterone. It is very likely that animals with an induced short luteal phase are infertile. To facilitate research in this area, we have initiated a program of collecting large quantities of porcine follicular fluid which will be made available to investigators.

Studies on Male Reproduction

Since the discovery of antifertility effects of gossypol by Chinese scientists, there has been a great interest in evaluating the effect of this chemical in a variety of *in vitro* and *in vivo* models. One of the difficulties experienced by scientists is the availability of pure gossypol. We are attempting to purify sufficient quantities of this chemical for in-house studies and for making it available to other scientists.

Natural Family Planning (NFP)

Primary activity in this area focuses on examination and analyses of available records in order to improve the understanding of the various menstrual cycle events and their influence on successful practice of NFP. Computer assisted interpretation of monthly charts appears to be of value in fostering improvement of teacher-client interactions.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Intrauterine Release of Estradiol for Contraception
Contract No. : NO1-HD-3-2729
Contractor : Michael Reese Hospital
Money Allocated: $133,233 (FY 74); $172,289 (FY 75); $107,760 (FY 76); $143,154 (FY 78); $47,419 (FY 79); $42,210 (FY 80)

Objectives: The objectives of this project are: (1) evaluation of the effects of an IUD releasing 12.5 µg/day of estriol in women; (2) evaluation of the long-term effect of intrauterine estriol on endometrial proliferation in baboons.

Major Findings: The clinical aspect of this study continues to progress slowly because of the difficulty in recruiting subjects who are willing to postpone their hysterectomies. It is hoped that a sufficient number of subjects will be recruited to finish the study in FY 81.

A baboon study to determine the effects of long-term intrauterine exposure to estriol is in progress and 6-month interval biopsies have shown no untoward results.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: Information desired on the pharmacodynamics of continuous administration of estriol should be available by the end of the current contract period. Further development is not projected at this time.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : A Study of the Use of Biodegradable Polymers for the Sustained Delivery of Contraceptive Drugs
Contract No. : NO1-HD-3-2741
Contractor : Research Triangle Institute
Money Allocated: $298,347 (FY 73 for two years); $185,262 (FY 75); $224,082 (FY 76); $211,983 (FY 77); $210,081 (FY 78); $306,740 (FY 79); $258,688 (FY 80)

Objectives: To develop a biodegradable polymer reservoir for subdermal delivery of a contraceptive steroid, e.g. norgestrel, for periods of one year or longer. The device is designed to provide constant release rate by diffusion control and to biodegrade after the drug is exhausted.

Major Findings: Pre-clinical studies necessary to obtain an IND for a 30-day clinical trial of a poly(ε-caprolactone)/levonorgestrel delivery system in 10 women had been completed and the IND was approved. After a substantial delay due to difficulty in obtaining product liability insured the study was initiated in late summer of 1980.

Studies of the effect of different formulations on the rate of release of levonorgestrel from production-grade poly(ε-caprolactone) capsules in vitro were continued. Using a 40:60 suspension of drug in ethyl oleate, the rate decreased gradually from 40 μg/cm/day to 15 μg/cm/day over a 382 day period. The use of a 1:1 mixture of levonorgestrel and cholesterol suspension as a 20% suspension in ethyl oleate resulted in a slightly lower release during the 240 days of the study. Methyl pentadecanoate behaved similarly to ethyl oleate as a suspending agent, while methyl tridecanoate was associated with a somewhat higher but declining release rate. The in vitro rate of release of levonorgestrel from capsules of star block copolymers of ε-caprolactone and δ-valerolactone was more rapid than that achieved with poly(ε-caprolactone) capsules of comparable dimensions, although the higher rate was not sustained.

In vitro measurements of the change in weight, crystalline and amorphous contents, and M.W. of star and linear block copolymers of ε-caprolactone and δ-valerolactone as a function of time in formic acid showed similarities in their behavior. The increase in crystallinity of random copolymers was intermediate between poly(ε-caprolactone) and the block copolymers, but in all cases the crystalline phase of the copolymers persisted during the later stages of weight loss.

Significance to Biomedical Research and Program of the Institute: Development of drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Evaluation of a Biodegradable Drug Delivery System
Contract No. : NO1-HD-4-2802
Contractor : Arthur D. Little, Inc.
Money Allocated: $60,900 (FY 74); $195,625 (FY 75 for 2 years); $161,628 (FY 77); $144,764 (FY 78); $93,624 (FY 79)

Objectives: The goal of this program is the development of implantable release systems that administer levonorgestrel at a rate of 50 to 100 micrograms per day for approximately six months and that biodegrade within twelve months.

Major Findings: Drug delivery systems have been developed in the form of rods composed of a 50/50 mixture of drug and polypeptide. The polypeptides were copolymers of glutamic acid and ethyl glutamate. These copolymers appeared to be biocompatible, nonantigenic, and completely biodegradable. Studies involving radiolabeled copolymers revealed that the degradation product was glutamic acid, and this amino acid ultimately entered the metabolic pool.

The glutamic acid content of the copolymer determines its moisture sorption and biodegradation rates - two properties that influence levonorgestrel release. A copolymer containing approximately 20 mole percent glutamic and exhibited the best balance between control of release and biodegradation.

It would appear that these systems can provide the target release of 50 µg/day of levonorgestrel. The biocompatibility of the copolymers and their ability to be degraded completely to L-glutamic acid make them useful for implantable drug delivery devices.

Significance to Biomedical Research and Program of the Institute: Development of drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This project will not be supported further since alternative approaches are more promising.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Antifertility Compounds. Novel Compounds Structurally Related to Steroids
Contract No. : NO1-HD-4-2846
Contractor : Oklahoma State University
Money Allocated: $17,575 (FY 74); $11,155 (FY 75); $39,971 (FY 76); $78,863 (FY 77 for eighteen months)

Objectives: The purpose of this project is to synthesize analogs of contraceptive steroids in which the B/C rings have been separated by a four-membered ring. These compounds will be tested for the antifertility activity.

Major Findings: Attempts to synthesize cyclobuta-BC-estrone were completely unsuccessful. None of the cyclobuta-BC estrone intermediates submitted for testing showed significant biological activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contracpetive utility.

Proposed Course: The contract was terminated owing to the difficulties encountered during the Ring D modification as well as angular methylation at C-13.

NICHD ANNUAL REPORT
October 1, 1979 through Septmeber 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of a Long-Acting Injectable Contraceptive
Contract No. : NO1-HD-5-2817
Contractor : Schering AG
Money Allocated: $1,211,168 (FY 75) 3 years; $120,000 (FY 78); $142,477 (FY 79); $88,782 (FY 80)

Objectives: The purpose of this contract program is to acquire sufficient data to support the filing of a New Drug Application (NDA) for norethindrone enanthate, a long-acting progestational agent. This requires support of preclinical, clinical and long-term drug safety studies.

Major Findings: Representatives of the sponsor (Schering AG), the World Health Organization (WHO), NIH and consultants presented preclinical and clinical data at the September 27, 1979 meeting of the Fertility and Maternal Health Drugs Advisory Committee to the Bureau of Drug, FDA. The Advisory Committee unanimously recommended approval of continued clinical trials and saw no need for the initiation of new safety studies (Long-term dog and monkey studies are in their fifth year with no unusual findings). On June 11, 1980, the FDA informed the sponsor that clinical trials could be initiated if a two year toxicity study comparing norethindrone enanthate with a combination oral contraceptive was instituted. Because of this unprecedented requirement, sponsor and the project officer will meet with members of the FDA in mid July to discuss the reason for this request and the precise nature of the study design envisioned by the Agency.

Data from metabolic studies and serum norethindrone levels obtained during Phase I clinical trials are in the process of being analyzed.

Investigators were not successful in developing a radioimmunoassay for the enanthate ester.

Significance to Biomedical Research and Program of the Institute: The development of a long-acting injectable contraceptive is directly related to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort encompassing completion of all preclinical and clinical requirements for the filing of a New Drug Application (NDA) and long-term drug safety studies in dogs and monkeys.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Potential Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-6-2843
Contractor : University of Colorado Medical Center
Money Allocated: $59, 348 (FY 76); $39,782 (FY 77); $45,839 (FY 78); $91,230 (FY 79)

Objectives: The goal of this project is to develop new highly potent antagonist analogs of LHRH.

Major Findings: Of the twenty-eight new LHRH analogs that were synthesized, two have been found to be among the most potent anti-ovulatory agents known. They are able to block ovulation in the rat when injected in a single administration at noon on the day of proestrus with an ID_{50} of approximately 5 µmg per rat. These compounds are [Acetyl-3,4-dehydroproline^{1},D-p-chloroPhe^{2},D-Trp^{3,6}] LHRH and [Acetyl-Ala^{1},D-p-ChloroPhe^{2},D-Trp^{3,6}]LHRH. The latter analog is very potent in the isolated rat hemipituitary in vitro assay, the male rat LH release assay and also in chimpanzees. A significant advance in understanding the receptor-bound conformation of LHRH has been made. A cyclic antagonist was synthesized having restricted conformation and it shows good potency. This is the first high potency cyclic analog. It is [Acetyl-D-Ala^{1}, D-Phe^{2}, D-Trp^{3,6}, Cys^{4,10}]LHRH in which the disulfide ring linking positions 4 and 10 drastically limits the peptide conformations available at the receptor.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LHRH analogs for contraceptive utility.

Proposed Course: This work is being continued under contract NO1-HD-0-2832.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Formulations for Improving Efficacy of Orally Administered Natural Steroids
Contract No. : NO1-HD-6-2847
Contractor : Arthur D. Little, Inc.
Money Allocated: $217,978 (FY 76); $253,130 (FY 77); $272,544 (FY 78); $217,954 (FY 79)

Objectives: It is proposed to increase the bioavailability of orally administered estradiol. The formulations are based on mixed lipid micelles which are the product of enzymatic hydrolysis of fat plus components of bile.

Major Findings: Results to date indicate that a mixed lipid micelle of 17β-estradiol (E_2) results in a slow and prolonged absorption of estradiol via the lymphatic pathway in both dogs and monkeys. In both species the areas under the curve for total estradiol equivalents are comparable to a molar equivalent intravenous dose; however, the peak concentration from the mixed lipid formulation is nearly an order of magnitude less than the intravenous dose.

A comparison of the total estradiol equivalents from 17β-estradiol administered by gavage in mixed lipid micelles and in 20% ethanol/saline indicates that the pattern of equivalents from mixed lipid micelle administered E_2 is more similar to the equivalents from the intravenously administered E_2 than those from the ethanol/saline administered E_2. Further, the peak concentration of total equivalents from the mixed lipid micelle formulated E_2 is approximately half that from the ethanol/saline administered E_2. These differences in the pattern of equivalents and peak concentrations may be of sufficient magnitude such that the pharmacological response of E_2 in a mixed lipid micelle would either be better or worse, but not comparable to that of E_2 administered in ethanol/saline.

Rat uterotropic assays of both formulations as well as assays of the mixed lipid micelle formulations in a monkey withdrawal bleeding protocol indicate that E_2 in the mixed lipid micelle formulation was slightly more pharmacologically active.

Significance to Biomedical Research and Program of the Institute: Increasing the bioavailability of orally administered natural steroids offers the potential of increased safety over the synthetic derivatives. Development of safe and efficacious contraceptives is the prime goal of the Contraceptive Development Branch.

Proposed Course: Although the mixed lipid micelle formulation might offer some advantage over current technology with respect to its safety, its biological activity is not sufficient to justify the costs associated with preclinical toxicological evaluation.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Biological Testing of Luteolytic Prostaglandin Analogs
Contract No. : NO1-HD-7-2816
Contractor : University of Chicago
Money Allocated: $137,214 (FY 77); $134,539 (FY 78); $142,000 (FY 80)

Objectives: The major objectives of this project are: 1) To synthesize 10, 10-difluoro-prostaglandin analogs for evaluation as luteolytic agents in the monkey; 2) Evaluate previously synthesized 16-fluoro-13-dehydroprostaglandin analogs for luteolytic activity and smooth muscle activity in the monkey.

Major Findings: In the course of synthesizing pure 10,10-difluoro-13-dehydro-$PGF_{2\alpha}$ and its diastercomer, ent-15-epi-10,10-difluoro-13-dehydro-$PGF_{2\alpha}$, it was found the assignments of absolute configuration on the basis of the Horeau and Mosher empirical rules were incorrect. This represents the first instance in which the effect of a CF_2 could be evaluated versus CH_2 in the Horeau and Mosher rules. Undoubtedly, the electronegativity of the former, which is sterically essentially equivalent to a CH_2 group, must be responsible for the reversal of the assignments predicted by the two rules.

The hCG mid-luteal phase monkey appears to be a good model for testing luteolysis. The doses of analog necessary to induce luteolysis are somewhat higher than those required to induce luteolysis in the cyclic monkey. While the 13-dehydro-$PGF_{2\alpha}$ was active at about 1.0 mg/kg/hr-6 hrs. i.v., the 16-fluoro analog was active in an amount 2 to 4 fold less. The ent-15-epi-13-dehydro-$PGF_{2\alpha}$ on the other hand was much less active although it should be noted that it was tested in the presence of much higher doses of exogenous hCG. The finding that very few side effects were observed with both the fluorinated and non-fluorinated 13-dehydro analogs is in keeping with the previous conclusion that the receptors controlling the luteolytic effect on the one hand and the smooth muscle effect on the other, possess different structural specificities. The possibility therefore remains open that analogs of even greater selectivity will be developed. Similar conclusions have been drawn recently by other investigators using different types of analogs (Bartman, et al, Prostaglandins 19, 301, 1979; Forbes & Wilks, Endocrinology 104, A726, 1979).

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2824
Contractor : University of Utah
Money Allocated: $124,612 (FY 77); $219,750 (FY 78); $199,223 (FY 79)

Objectives: The goal of this research proposal is to synthesize and characterize biodegradable polymers of glutamic acid to which a progestin is covalently bonded. The polymer system should be capable of delivering drug by means of hydrolytic cleavage in vivo for 6-12 months.

Major Findings: Norethindrone has been successfully bonded to poly[N^5-(3-hydroxypropyl)-L-glutamine] via a 17-carbonate ester linkage (Compound XXb). Pilot studies involving bonding of norethindrone to poly[N^5(3-hydroxyethyl-L-glutamine] also via the 17-carbonate ester linkage (Compound XXa) also have been successful. Reaction of norethindrone-3-oxime with the free carboxy groups of poly-(L-glutamic acid) to form the esterified compound (Compound XXII) has been completed. Compound XXb has been obtained as a powder in various particle sizes ranging from approximately 10μ to about 150 μ. Methods have also been developed for controlled variation in the per cent of loading of norethindrone onto available reactive sites on poly[N^5-3-hydroxypropyl)-L-glutamine]. In vitro evaluation of release rates has shown good correlation with in vivo results and batch-to-batch consistency. In addition norethindrone(3-N,N-dimethylamine)propyl carbonate to determine the stability of the carbonate linkage of the potential hydrolysis products.

In vivo testing has revealed a correlation between release rate and particle size, however, it is not directly proportionate to surface area. The release rates were not dependent on extent of loading over the range investigated (28-42%). Preliminary data on the effect of the length of the spacer group indicate that decreasing the length of the group, i.e. propyl to ethyl decreases the release rate. Finally, the oxime linkage appears to give a higher release rate than the carbonate linkage.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: Although some promising leads have evolved from this work, the large costs of preclinical toxicological testing coupled with limited program sources and other more advanced projects have resulted in discontinuation of support of this project after the current contract expires.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2825
Contractor : ALZA Corporation
Money Allocated: $206,289 (FY 77); $204,284 (FY 78); $455,411 (FY 79); $356,612 (FY 80)

Objectives: The goal of this project is the development of a biodegradable, implantable delivery system that will deliver predictable, contraceptive amounts of synthetic progestins over a four to six month period.

Major Findings: The CHRONOMER™ - norethindrone drug delivery system which had shown local irritation in several animal models was extensively investigated. A complex between polymer and drug after sterilization by γ-irradiation has been discovered. The exact nature of this complex which definitely is covalently linked has not been determined nor has a correlation between the observed irritation and the existence of the complex been established.

Work continued on the formulation for the CHRONOMER™ - norgestrel system. After narrowing down the potential base excipients to MgO and Na_2CO_3, it was determined that Na_2CO_3 which was used in the norethindrone system appeared as good if not better than MgO. Thus, this formulation will be the final one and will be tested in a 10-month study in baboons.

Disposition studies on the potential hydrolysis products of the polymer, i.e. 1,4 cyclohexane dimethanol and γ-butyrolactone, have begun in rats using radiolabelled compounds. In addition, radiolabelled polymer has been prepared and after coating on a thin rod to approximate a burst of erosion, it is also being studied in rats. So far no significant defference in distribution and excretion has been noted.

The scale-up of CHRONOMER™ production from 100g batches to 1 kg batches has been successfully carried out.

Significance to Biomedical Research and Program of the Institute: Development of new drug delivery systems for contraceptive drugs is one of the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2826
Contractor : SRI International
Money Allocated: $149,786 (FY 77); $237,555 (FY 78); $270,465 (FY 79)

Objectives: It is proposed to develop non-toxic biodegradable polymers capable of delivering a contraceptive agent with zero order kinetics over a six-month period. Additionally, drug release and polymer solubilization should take place concomitantly so that rate of drug release is controlled by the rate of polymer dissolution.

Major Findings: (1) A poly(ortho ester) matrix system has been developed using a novel synthetic route, which can be satisfactorily compounded with substantial quantities of solid inorganic buffer salts and steroids; (2) these matrices, as described above, have been shown to release drug at a constant rate through surface erosion resulting from polymer hydrolysis and a patent application is pending on this invention; (3) a hypothesis has been proposed for the mechanism of drug release; (4) a polyacetal matrix system, produced by a synthetic route similar to that for the polyesters, has been studied and appears to release drug through a diffusional mechanism coupled with bulk polymer degradation.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : The Synthesis and Testing of Polymeric Intravaginal Contraceptives
Contract No. : NO1-HD-7-2828
Contractor : SRI International
Money Allocated: $68,050 (FY 77); $28,871 (FY 78); $12,000 (FY 79)

Objectives: The objective of this project is to synthesize relatively large polymeric intravaginal contraceptives in order to decrease the absorption of the contraceptive agent across the vaginal barrier (by increasing their molecular weight), and thereby preventing systemic side effects as well as lengthening the period of potency.

Major Findings: SRI successfully synthesized four polymers, N-(methoxy polyethylene oxide)ethyl maleimide, copoly(N-vinylpyrrolidinone - vinylthiol), polyvinylmethylamine hydrochloride, and copoly[vinylmethylamine - vinylmethyl (3-mercapto-1-succinyl)amine], and three monomeric analog models, N-2-(2'-methoxyethoxy)ethyl malemide, N-2-(2'-methoxythoxy)ethyl acrylamide, and 17-hydroxy-17α-[N-2-(2'-methoxyethoxy)ethyl)carboxamidoethynyl]-5(10)-estren-3-one. Polyacrylic acid was obtained and purified.

The polymeric sulfhydryl group reagent N-(methoxy polyethylene oxide)ethyl maleimide was inactive in an *in vitro* immobilization assay with human spermatozoa. Of the two polymeric disulfide group reagents prepared, copoly(N-vinylpyrrolidinone -vinylthiol) and copoly[vinyl-methylamine -vinylmethyl (3-mercapto-1-succinyl)amine] only the former showed weak activity in the *in vitro* assay; this activity was evident only in the presence of a detergent. Apparently, the functional sulfhydryl and disulfide groups of the spermatozoan plasmalemma membrane are not readily accessible to these polymeric reagents.

The polymeric acidic buffers, polyvinylmethylamine hydrochloride and polyacrylic acid, displayed good spermicidal activity. These polymers lowered the pH of the medium to 5, at which the sperm were immobilized. Because the pH of the human vaginal tract is acidic (3.5-5.3), a polymeric acidic buffer should be suitable in such a medium as a spermicidal agent.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new intravaginal contraceptives.

Proposed Course: Termination. All of the workscope objectives have been completed.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Development of a Radioimmunoassay for Rhesus Monkey Luteinizing Hormone
Contract Number	:	NO1-HD-7-2829
Contractor	:	University of Pittsburg
Money Allocated	:	$66,331 (FY 77); $65,160 (FY 78); $91,664 (FY 79); $108,160 (FY 80)

Objectives: The aim of the program is to develop a radioimmunoassay for rhesus monkey luteinizing hormone (LH) and to provide sufficient reagents to prepare 200 kits (15,000 tubes per kit) for distribution to the scientific community.

Major Findings: Purification of LH from cynomolgus glands has been completed and characterized. By the mouse Leydig cell testosterone production assay, this cynomolgus LH preparation (WP-XV-63-2429) showed a potency of 9.84 relative to that of rhesus LH standard WP-XIII-127-21. This compares quite favorably with the cynomolgus preparation's relative potency of 10.6 by LH RIA in the rhesus: anti-hCG 267 system and is consistent with a high degree of purity for this preparation.

It appears that the antiserum used in the final version of the monkey LH RIA will probably be anti-hCG R13. A volume on hand to date is only 90 ml. This anti-serum, however, is used at a final dilution of 1:100,000, and consequently the 90 ml should be enough for 9 million tubes, or 600 assay kits. Therefore the quantity of antisera will not be the limiting factor in the number of RIA kits which can be assembled. On the other hand, if each kit includes 25 µg of highly purified cynomolgus LH, a reasonable but probably near minimal amount, the present supply of this purified hormone would provide for only about 250 kits.

Partially purified cynomolgus LH for reference preparation is available in sizeable quantity, i.e., about 30 mg. equivalents of NICHD-WDP-rhLH-RPI, or approximately 3/4 the amount of LH which was provided for the NICHD rhesus standard. This material has not been yet tested rigorously to determine its suitability as a reference preparation, but its chromatographic behavior suggests that contamination with LH beta subunit will not be a problem.

Significance to Biomedical Research and Program of the Institute: The work to be undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to acquire specific substances concerned with aspects of reproduction processes that are subject to experimental interventions.

Proposed Course: This is expected to be completed by the end of the contract year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Orally Active Sustained Release Dosage Forms for Synthetic Steroids
Contract No. : NO1-HD-7-2830
Contractor : The University of Kentucky Research Foundation
Money Allocated: $101,500 (FY 77); $102,000 (FY 78); $25,500 (FY 79)

Objectives: It is proposed to develop a sustained release dosage form which will release norethindrone and ethynylestradiol at such a rate that effective plasma levels of these synthetic steroids will be maintained for up to 24 hours.

Major Findings: Ethynylestradiol and norethindrone have been formulated in sustained release tablets using FDA approved tablet excipients. Three different strength sustained release tablets were prepared for each steroid: 0.035 mg, 0.018 mg, and 0.009 mg for ethynylestradiol, and 0.5 mg, 0.25 mg, and 0.125 mg for norethindrone. The dissolution profiles for the ethynylestradiol tablets were identical and independent of the content of ethynylestradiol. The same results were found for the norethindrone tablets. The effect of pH (pH 1.3-7.4) on the rate of release of the steroids from the sustained release tablets was found to be very small.

In vivo studies using beagle dogs showed that ethynylestradiol is subject to extensive first-pass metabolism and the bioavailability as determined from the comparison of the area under the curve of unchanged steroid after oral and IV administration is less than 6%. The results from administration of different doses indicated the first-pass metabolism of ethynylestradiol is dose dependent suggesting the possibility of a saturable metabolism.

Norethindrone was not metabolized as extensively as ethynylestradiol on the first pass after oral administration and the availability was found to be 70-80% compared to IV administration. Furthermore, the area under the plasma level curve was found to be directly proportional to the dose of norethindrone indicating dose independent metabolism.

Long term stability studies at room temperature and 37°C showed that although the steroids are stable, the release rate of the steroids from the tablets decreased upon storage.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project was directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: The original objectives of this project have been accomplished. It is hoped that future development of this approach will be pursued in the private sector.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Orally Active Dosage Forms for Steroids
Contract No. : NO1-HD-7-2831
Contractor : Southwest Research Institute
Money Allocated: $95,027 (FY 77); $104,104 (FY 78); $323,500 (FY 79); $280,883 (FY 80)

Objectives: The aim of this program is to prepare sustained-release oral formulations of ethynylestradiol (EE) and norethindrone (NET), by incorporating these products in a matrix of microcapsule and to determine whether this dosage form will minimize daily peaks of the drugs and reduce body burdens while maintaining contraceptive efficacy and clinical acceptability.

Major Findings: Studies have continued investigating the development and evaluation of sustained-release microspheres containing NET or EE in matricies of glycerides, fatty alcohols, fatty acids and mixtures thereof. Microspheres of EE in the glycerides have been shown to be stable with very little change in the in-vitro release rate up to four months at 37°C. In the case of NET-containing glyceride microspheres, decrease in release rate is observable after two weeks' aging at 37°C. This phenomenon appears to be due to the crystallization of the steroid in the glycerides because of low solubility. To resolve the aging problem, microspheres of NET have been prepared using various fatty alcohols as the matrix, as NET is known to be more soluble in alcohols. Aging results indicate the stability of these formulations at 37°C based upon in-vitro release rate studies. However, the formulations tested to date do not give the desired sustained-release. In an attempt to achieve both a satisfactory in-vitro release rate and aging stability, combinations of fatty alcohols and glycerides are being examined. Encouraging release rate data have been obtained for several formulations; however, most of the formulations do not have long term aging stability at 37°C. One formulation appears promising, i.e. a matrix of 80/20 fatty alcohol/triglyceride which has shown stability after aging at 37°C for four months. Further studies are in progress to determine longer term stability and reproducibility of results.

In-vivo investigations with some of the NET-containing fatty alcohol microsphere were conducted. In the case where the microsphere excipient content was nearly 100% fatty alcohols, low absorption of the steroid was observed, and sustained-release was not demonstrated. Mixtures of the fatty alcohols with the glycerides gave improved sustained-release; however, the absorption is still lower than that observed with microspheres using the glycerides alone as the matrix.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Folliculogenesis in the Rhesus Monkey During the Menstrual Cycle
Contract Number: NO1-HD-7-2835
Contractor : George Washington University
Money Allocated: $45,999 (FY 77), $22,204 (FY 78), $57,818 (FY 80), $4,822 (FY 81)

Objectives: The objective of this contract is to determine the distribution of preantral follicles in serially sectioned rhesus monkey ovaries. It is anticipated that some waves of follicular growth and atresia will be identified at specific times during the menstrual cycle.

Major Findings: The investigator has in essence completed the original workscope dealing with the status of preantral follicles during the menstrual cycle of the rhesus monkey. Nine pairs of ovaries have been analyzed and all preantral follicles on every twentieth section have been classified. Preantral follicles with diameters of 50 and 60 μm are more prevalant during the follicular phase. This suggests that preantral follicles have a growth pattern that may be in synchrony with the one seen in antral follicles.

Another observation that may also be of major importance is the manner in which a great number of follicles develop. Preantral follicles appear in all ovaries in clusters. Each of these groups consists of follicles of varying sizes. This observation suggests that adjacent follicles which are in a similar environment are either sensitive to stimulation at different times or they grow at different rates -- a situation quite similar to the one observed with antral follicles.

Atresia of preantral follicles can be identified by involution of the ovum and compactness of the granulosa cells. This stage is easily identified and is prevalent in some animals but shows no relationship to the cycle.

Polyovular follicles were observed in ovaries of mature monkeys and their presence cannot be utilized as a criterion of animal's age.

Significance to Biomedical Research and Program of the Institute: Since the ovarian follicle is the target for contraceptives, the knowledge of its natural history is important for appreciation of the possible mechanisms of action of contraceptives.

Proposed Course: The contract was extended to permit additional observations. Research will be completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Contraceptive Efficacy of Androgen-Estrogen Polydimethylsiloxane Implants in Rhesus Monkeys
Contract Number: NO1-HD-8-2805
Contractor : The John Hopkins University
Money Allocated: $70,937 (FY 78), $163,604 (FY 79), $96,217 (FY 80)

Objectives; The overall goal of this study is to design a reversible contraceptive technique for the human male which does not alter sexual behavior, accessory sex organ function or result in untoward side effects. The immediate objects of this proposal are twofold: (1) To define the most efficacious contraceptive formulation of testosterone and estradiol delivered via PDS subdermal implants to rhesus monkeys; and (2) to test the effect of this contraceptive formulation on fertility and erythropoietic system, blood clotting mechanisms and major organ function in rhesus monkeys.

Major Findings: Maximum suppression of sperm per ejaculate was obtained in monkeys treated with 86 or 100 µg T/kg/day plus 0.48, 0.72 and 0.96 µg E_2/kg/day. For example, sperm per ejaculate was 2-4% of controls. Most treated animals were azoospermic but some were oligospermic during this period. Seminal plasma volume was similar to controls in these treated monkeys. Thus the most efficacious T-E_2 dose is between 86-150 µg T and 0.48-0.72 µg E_2/kg/day. Peripheral serum concentrations of T and E_2 were slightly elevated above the controls in these treated monkeys. For example, serum T concentration was 7.3 ± 2.4, 12.3 ± 0.9 and 12.4 ± 1.0 ng/ml in control, 86 µg T + 0.48 µg E_2 and 86 µg T + 0.72 µg E_2/kg/day. Serum E_2 concentration was 20 ± 2.9,53 ± 8.5 and 35 ± 7 pg/ml for control, 86 µg T + 0.48 µg E_2 and 86 µg T + 0.72 µg E_2/kg/day. Rhesus monkeys have a pronounced diurnal variation in serum T concentration with a peak at 0400 and a nadir at 1100 hrs in a colony where lights are on at 0700 and off at 1900 hrs. Serum samples from monkeys were always collected at 0900-1100 hrs. Thus the testosterone and perhaps estradiol concentrations reported herein for control monkeys may be the lowest which occurs in male monkeys in the colony. It is probable that the diurnal variation in serum T in the T+E_2 treated monkeys has been damped or eliminated because the bulk of serum T and E_2 will be derived from the subdermal silastic implants. This is probably the case because the seminal plasma volumes (which reflect accessory sex organ secretion) were identical in control and T+E_2 treated monkeys.

Significance to Biomedical Research and Program of the Institute: Development of new techniques for the control of fertility in the male is one of the stated goals of the program.

Proposed Course: These studies are expected to be completed at the end of the contract year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Postovulatory Prostanoid Antifertility Agents
Contract No. : NO1-HD-8-2808
Contractor : University of Washington
Money Allocated: $50,039 (FY 78); $51,887 (FY 79)

Objectives: The major objectives of this project are to synthesize bis-trifluoromethylated prostaglandins to obtain the beneficial effect of gem-dimethylation (a conformational effect) and fluorination (an electronic effect).

Major Findings: It has been established that the synthesis of bistrifluoromethylated $PGF_{2\alpha}$ analogs cannot be accomplished using standard PG synthesis methodology. During the past year, the required synthetic intermediates for novel routes have been prepared.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This project will be completed under contract NO1-HD-0-2814.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Biological Evaluation of Luteolytic Agents
Contract No. : NO1-HD-8-2809
Contractor : University of Pittsburgh
Money Allocated: $99,740 (FY 78); $71,812 (FY 79)

Objectives: The objectives of this project are a) to synthesize 100 mg of optically active (+)-12α-fluoro-$PGF_{2\alpha}$ and to determine its luteolytic and uterine contracting activity in the monkey and b) to synthesize several fluoroprostaglandins substituted at the 10-, 12-, and 14-positions and to determine their antifertility effects in hamsters as well as their oxytocic and smooth muscle activities.

Major Findings: During the past year the total syntheses of (+)-13(Z)-14-fluoro$PGF_{2\alpha}$ methyl ester (1), (+)-13(Z)-15-epi-14-fluoro$PGF_{2\alpha}$ methyl ester(2), (±)-13(E)-12,14-difluoro$PGF_{2\alpha}$ methyl ester (3), (±)-13(E)-15-epi-12,14-difluoro $PGF_{2\alpha}$ methyl ester (4), and (+)-12-fluoro$PGF_{2\alpha}$ were completed. In addition, 125 mg of (+)-12-fluoro$PGF_{2\alpha}$ methyl ester (6) was prepared for biological evaluation in the monkey. The results continue to demonstrate that replacement of a proton on the prostaglandin skeleton by a fluorine atom results in suppression of smooth muscle stimulating activity while at the same time maintaining "luteolytic" potency. Results obtained with both (+)-13(Z)-14-fluoro$PGF_{2\alpha}$ methyl ester (1) and (+)-13(Z)-15-epi-14-fluoro$PGF_{2\alpha}$ methyl ester (2) indicate that each analog was luteolytically effective in hamsters (25 µg) subcutaneously. Compound 1 possessed 26-28% the potency of natural $PGF_{2\alpha}$ in the hamster uterine strip assay and 2.3-3.7% the potency of natural $PGF_{2\alpha}$ in the isolated gerbil colon assay. Compound 2 was even more encouraging possessing only 2.5% the activity of $PGF_{2\alpha}$ in the hamster uterine strip assay and only 0.34-0.41% the activity of $PGF_{2\alpha}$ in the gerbil colon assay. With regard to the antifertility data on racemic 13(E)-12,14-difluoro-$PGF_{2\alpha}$ methyl ester (3), the minimum effective dose was 6.0 µg. The smooth muscle assays revealed that 3 possessed 12.8% the potency of $PGF_{2\alpha}$ in the hamster assay and only 1.3-2.4% the activity of $PGF_{2\alpha}$ in the gerbil colon assay. In contrast racemic 13(E)-15-epi-12,14-difluoro$PGF_{2\alpha}$ methyl ester (4) was ineffective at a dose level of 6.0 µg in the antifertility assay. This was somewhat disappointing in view of the low smooth muscle stimulating activity (2% of the activity of $PGF_{2\alpha}$ in both assays). The most interesting observation recorded was with (+)-12-fluoro$PGF_{2\alpha}$ (5). The minimum effective luteolytic dose was 0.5 µg which revealed that analog 5 is some 2 times more potent than (+)-12-fluoro$PGF_{2\alpha}$ methyl ester (6) and 25 times more potent than natural $PGF_{2\alpha}$. Smooth muscle data revealed 5 possessed 57-73% the potency of $PGF_{2\alpha}$ in the hamster assay and 18-28% the activity of $PGF_{2\alpha}$ in the gerbil colon assay.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This project will be continued on contract NO1-HD-0-2813.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Inhibition of Progesterone Biosynthesis and Antiprogestational Agents
Contract No. : NO1-HD-8-2810
Contractor : SRI International
Money Allocated: $165,813 (FY 78); $143,239 (FY 79)

Objectives: The objective of this project is to synthesize a) inhibitors of progesterone biosynthesis, (e.g., inhibitors of 3β-hydroxysteroid dehydrogenase and Δ^5-3-ketosteroid isomerase) and b) competitive inhibitors of progesterone as represented by certain ring-D modified steroids.

Major Findings: Based on previous structure activity studies and computer generated model studies several 15,16-seco derivatives were selected for synthesis as potential progesterone antagonists. The synthesis of target seco compounds 15,16-seco-19-norandrosta-4-en-3,17-dione, 17β-hydroxy-17α-ethynyl-15,16-seco-19-norandrosta-4-en-3-one and 17α-hydroxy-17β-ethynyl-15,16-seco-19-norandrosta-4-en-3-one and a large supply of the key intermediate 3-hydroxy-15,16-secoestra-1,3,5(10)-trien-17-one 3-methyl ether have been completed. The synthesis of remaining target seco compounds are currently underway. The initial analog showed binding affinity of 40-105% relative to that of progesterone depending upon the time (2 hrs./105% in 24 hrs./40%) of the measurement. In vivo, the analog exhibited about 4-5% of the progestational activity of progesterone. This leads to the concept that these analogs can be further modified to retain good binding affinity with the elimination of progestational activity to provide us with an antiprogestational agent. This appears to be be the first example of high progesterone binding affinity and any Clauberg activity associated with an incomplete steroid skeleton.

Based on previous structure activities 2,3-d-isoxazole androstane derivatives that have 4,4-dimethyl groups and varying C-17β side chains were synthesized. These side chain modifications included a 17β-acetamido, 17β-hydroxy-17α-ethynyl, 17β-acetyl and 20-methylene pregnane analogs. The synthesis of target compounds 4,4-dimethyl-17β-hydroxy-17α-ethynyl androst-5-en-[2,3-d]isoxazole, 17β-acetamido-4,4-dimethyl-androst-5-en-[2,3-d]-isoxazole, 4,4-dimethyl-20-methylene-pregn--5-en-[2,3-d]isoxazole, have been completed. The target compounds were assayed for estrogen, progesterone and androgen cytosol receptor binding activity as well as for dehydrogenase-isomerase and 17,20-lyase enzyme inhibitor activity. These 2,3-d-isoxazole derivatives were also submitted for *in vivo* assays for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: Since the workscope objectives have been accomplished, a new competitive RFP will be issued to explore further development of this project.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Antiovulatory and Postovulatory Agents
Contract No. : NO1-HD-8-2811
Contractor : Research Triangle Institute
Money Allocated : $152,781 (FY 78); $163,280 (FY 79); $22,421 (FY 80)

Objectives: The objective of this contract is to synthesize potent aromatase inhibitors in order to block implantation, and to develop compounds which may be antiprogestational agents, possibly through inhibition on progesterone biosynthesis. These compounds will be tested for antifertility activity.

Major Findings: Seven compounds, 17-oxa-D-homoandrost-4-ene-3, 16-dione, 17-oxa-D-homoandrosta-4,6-diene-3,16-dione, 3β-acetoxy-17-oxa-D-homoandrost-5-en-17a-one, 17-oxa-D-homoandrosta-1,4,6-triene-3, 17a-dione and 17-oxa-D-homoandrost-4-ene-3,6,17a-trione have been prepared as potential aromatase inhibitors and tested for various types of biological and biochemical activity. Particularly, the last three lactones exhibited 37-46% aromatase inhibition in vitro relative to the standard compound androsta-1,4,6-triene-3,17-dione. The standard compound showed also ovarian aromatase inhibition in the rat after subcutaneous administration and blocked implantation when given in very large sustained doses on days 2,5 and 9 of pregnancy. In addition it was completely effective in inhibiting ovulation in the rat at a subcutaneous dose of 3 mg/g and 10 mg/kg b.i.d. given on metestrus, diestrus, and proestrus. 17-oxa-D-homoandrosta-1,4,6-triene-3,17a-dione was also effective in inhibiting ovulation in the rat at a subcutaneous dose of 30 mg/kg b.i.d. given on metestrus, diestrus, and proestrus, suggesting that this lactone is about 1/10th as potent as the standard compound in suppressing ovulation. All lactones synthesized and tested did not possess any binding affinities for estrogen, progestin, and androgen receptors, and were completely devoid of estrogenic activity.

Significance to Biomedical Research and the Program of the Institute: The work to be undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of usual modifications of steroids for contraceptive utility.

Proposed Course: Termination in the absence of significant biological activity for these compounds.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Progesterone Analogues of Novel Structures with Potential Antifertility Activities
Contract No. : NO1-HD-8-2812
Contractor : Laval University
Money Allocated: $74,715 (FY 78); $64,784 (FY 79)

Objectives: The objective of this contract is to synthesize derivatives of 17α-hydroxymethylprogesterone and 11α-substituted progesterone analogues in view of the potentiating effect of 17-alpha substituents and the influence of substitution at C-11 on progestational activity. These compounds will be tested for antifertility activity.

Major Findings: 17-Hydroxymethylprogesterone was prepared and converted to the corresponding tosylate. Transformation of this tosylate into the 17-fluoromethyl and methoxymethyl analogs of progesterone is in progress. It was found that the replacement of the tosyl group by fluorine was not readily achieved in the presence of an unprotected 20-keto group. It was necessary to prepare the protected 3,20-bisethylenedioxy-17-hydroxymethyl-5-pregnene by the published procedure as described in Can. J. Chem. 56, 410 (1978) as well as the corresponding tosylate. It will be used for the preparation of 17-fluoromethyl and methoxymethyl analogs of progesterone.

With regard to the synthesis of 3,20-dioxygenated-11-substituted progesterone, the 11α-bromo-12-ketone was treated with lithium dimethyl cuprate and subsequently methyl iodide. The major product, 3α,20β-diacetoxy-11-α-methyl-5β-pregnan-12-one was formed along with a small amount of the 11β-isomer. The 12-keto group was readily removed by Barton's modification of Huang-Minlon's procedure. 11α-Methyl-5β-pregna-3,20-dione was obtained by oxidation of the corresponding diol with Jone's reagent. As already reported, a small amount of the same compound had been obtained earlier from 3α,20α-diacetoxy-5β-pregnan 12-one. The 4-double bond was introduced by McGuickin & Kendall's method (J. Am. Chem. Soc., 74, 5811 (1952)) via the formation of the 4-bromide and the semicarbazone. At the present a very small amount of 11α-methyl progesterone is available for testing.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Specific Immunization Against FSH as a Male Contraceptive
Contract No. : NO1-HD-8-2814
Contractor : University of Colorado Medical Center
Money Allocated: $63,744 (FY 78); $69,268 (FY 79)

Objectives: The objective of this project is to produce specific anti-FSH sera to block spermatogenesis without effect on secondary sex characteristics and thus to develop an immunologically based male contraceptive.

Major Findings: The syntheses of all the proposed unique sequences of human FSH were completed, the sequences were conjugated to protein carriers and male rabbits were immunized with these immunogens. Most of the immunization schedules for these immunogens have been completed. All of the rabbits showed significant antibody production directed against the peptide immunogen, but so far none of these has showed significant cross-reactivity with intact FSH or the ability to immunostain rat anterior pituitary, nor have the rabbits shown any pathology which could be attributed to antagonism of FSH function. All of the proposed work used bovine serum albumin as carrier. This was recently extended to larger peptides and other protein carriers. Horseshoe crab hemocyanin is reported to be the most highly antigenic protein known, so certain peptides were conjugated to this carrier in the hope of changing the antibody specificity. Early bleedings from rabbits immunized with one such antigen, hFSH(94-118) coupled to horseshoe crab hemocyanin via the glutaraldehyde method show for the first time some cross reactivity with whole FSH. Also synthesized was hFSH(80-118), which contains five of the 12 crysteine residues in the FSH β-subunit. This peptide was oxidized in concentrated solution to make a disulfide polymer which may have a conformation more closely resembling that in the intact FSH molecule and thus be more likely to produce antibodies which will react with FSH.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the development of a specific immune-based male contraceptive.

Proposed Course: Termination. All of the workscope objectives have been completed.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Novel Steroids with Potential Male Contraceptive Activity
Contract No. : NO1-HD-8-2815
Contractor : Laval University
Money Allocated: $33,812 (FY 78); $37,178 (FY 79)

Objectives: The objective of this contract is to synthesize 11-aza-5α-dihydrotestosterone analogs which will lead to the study of a distinctly different 11-hetero steroid. The importance of the 11-position in governing both estrogenicity and effects on gonadotrophin is now apparent. These compounds will be tested for the male contraceptive activity.

Major Findings: Hydroxylation in position 17 of N-trifluoroacetyl-3,3-dimethoxy-11-aza-5α-pregnan-20-one was found to be unsuccessful in order to obtain 11-aza-dihydrotestosterone after degradation of the side chain of the former. Larger quantities of 3,20-dioxygenated-11-aza-5α-pregnan should be prepared for further exploration of the Barton's hydroxylation conditions and appropriate protection of the nitrogen group. Alternatively, these difficulties could be circumvented by the degradation of the side chain prior to the introduction of the 11-aza function. 3β-Acetoxy-17-hydroxy-5α-pregnan-12,20-dione prepared from hecogenin could be used as a starting material for the synthesis of a C-seco compound devoid of the 17-side chain, namely methyl 3-hydroxy-9,17-diketo-11-nor-9,12-seco-5α-androstan-12-oate. It is hoped that the transformation of this seco compound might lead directly to the desired 11-aza-dihydrotestosterone. A new supply of hecogenin appears to be an obstacle for this project.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaboration Research

Contract Title : Synthesis and Testing of Male Contraceptive Agents
Contract No. : NO1-HD-8-2816
Contractor : SRI International
Money Allocated: $42,271 (FY-78)

Objectives: The purpose of this project is to prepare potent orally active new androgens that will act on spermatogenesis by inhibiting gonadotropin and maintain libido and secondary sex characteristics because of their great potency. These compounds will be tested for the male contraceptive activity.

Major Findings: 11β-Hydroxy-7α-methyl-19-nortestoterone and 11β-methoxy-7α-methyl-19-nortestosterone were synthesized and were submitted for androgenic assay. The former was twice as potent as testosterone subcutaneously. However, the latter was less potent than testosterone subcutaneously.

Singnificance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: The objectives of the contract were completed.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Male Contraceptive Agents
Contract No. : NO1-HD-8-2817
Contractor : SRI International
Money Allocated : $22,450 (FY 78); $23,805 (FY 79)

Objectives: The objectives of this project are to synthesize potential antispermatogenic agents. The compounds to be synthesized are analogs of 4-(p-cholorophenylthio)butanol which was found to have antispermatogenic properties in rats and beagles during the course of toxicological examination.

Major Findings: The lead compound, 4-p-chlorphenylthiobutanol, was originally found to cause aspermic urine in rats and beagles at doses of 100-200 mg/kg over the course of 13-week toxicity evaluations. Animals taken off the regimen after 4 weeks showed totally aspermic urine at 8 weeks, with recovery by the end of 13 weeks. When the compound was reevaluated in the NICHD laboratories in the Male AFII assay, a daily dose of 200 mg/kg for 5-7 days had little effect on rats. However, at a dose of 500 mg/kg, a definite antispermatogenic effect was noted, but two animals died.

In an effort to improve on the potency and toxicity of the lead compound, eleven analogs were synthesized. These compounds have had substituent variation at the para position of the phenyl ring and incorporation of varying side chains attached to the sulfur atom. Para substituents have included chlorine, bromine, fluorine and lower alkyl such as methyl and t-butyl. Chain lengths have been shortened to three carbons and lengthened to five carbons (phenylthio-propanols to -pentanols). Two metabolites of the lead compound, namely the p-chlorophenylthiobutyric acid and the sulfone have been prepared.

Biological testing data obtained thus far suggest that activity is similar for the lead compound and p-t-butyphenylthiobutanol, while the p-methyl was inactive. Substitution of halogen at the p-position causes higher toxicity, especially for p-fluoro analogs. Shortening of the thio alkyl chain appeared to increase toxicity without improvement in antifertility effect. The effect of chain lengthening is still being evaluated. The butyric acid metabolite above was found to be inactive, while the sulfone is still in testing.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards the development of male contraceptive agents.

Proposed Course: Termination. All of the workscope objectives have been accomplished.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Analogs of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-8-2819
Contractor : Tulane University
Money Allocated: $156,691 (FY 78); $151,986 (FY 79)

Objectives: The objective of this project is to synthesize and biologically evaluate analogs of LHRH modified at various amino acid residues in order to produce very potent and long-acting antiovulatory agents.

Major Findings: In the latest, extremely active peptides, substitution by D-Ala in position 10 resulted in about a 2-fold increase in anti-ovulatory activity. The added stabilization of the C-terminus towards enzymatic attack must be considered an added advantage to the analogs. Thus, both [Ac-D-Trp^{1},D-p-Cl-Phe^{2}, D-Phe^{3},D-Phe^{6},D-Ala^{10}]-LH-RH and the corresponding Ac-D-p-Cl-Phe^{1} analog were highly active at the 5-10 µg dose level in a rat antiovulatory assay.

The great effectiveness of the D-p-Cl-Phe^{2}-modification, which was discovered during the first year of this contract, led to an intensive investigation of other substituents in the o-, m- and p- positions of the aromatic ring. In summary, o-substituents resulted in drastic loss of activity whereas substituents in the m- and p-positions generally retained appreciable activity, albeit less than the parent D-p-Cl-Phe^{2}-analog. F, Br, Me, OH, and NO_2 in the p-position were all inferior to Cl. The aromatic ring in position 1 (D-Phe) was much less sensitive to substitution. Substitution of Cl in the 3 positions generally retained high levels of activity and resulted in increased activity when in the p-position. Other groups in the p-position also retained activity, but in the main part were less effective than Cl. D-Trp in conjunction with D-p-Cl-Phe^{2} was particularly effective. D-Trp^{2}-compounds had severely reduced activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This project is being continued under contract NO1-HD-0-2831.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-8-2820
Contractor : University of Texas at Austin
Money Allocated: $196,973 (FY 78); $212,122 (FY 79)

Objectives: The objective of this project is to synthesize and biologically evaluate analogs of LHRH (particularly changes in the 1-, 3-, and 10- positions of [AA^1,D-Phe^2,AA^3,D-Trp^6]LHRH) in order to obtain very potent and long-acting antiovulatory agents.

Major Findings: The most potent LHRH antagonist emerging from this contract was [Ac-D-Thr^1,D-p-Cl-Phe^2,D-$Trp^{3,6}$,$Azgly^{10}$]LHRH. It completely inhibited the in vitro LHRH induced LH and FSH response at 3 ug and was 100% effective in vivo in the rat antiovulatory assay at 25 µg. However, the enhancement of activity with the $Azgly^{10}$ does not necessarily hold for other LHRH analogs. The most potent undecapeptide was [(N-Ac-Pro-Pro)^1,D-Phe^2,D-$Trp^{3,6}$]LHRH. It had 100% activity in vivo at 100 µg. A new type of analog, a tedradecapeptide, was fully active in vivo at 200 µg and in vitro at 100 ng. A final observation worthy of emphasis is that [N-Ac-L-Thr^1,D-Phe^2,D-$Trp^{3,6}$,D-Arg^8]LH-RH definitely had a greater inhibitory effect (30 ng and/or 100 ng) on the LH-RH LH response than on the LH-RH FSH response. Although these analogs had a weak antiovulatory activity (>200 µg), these results may indicate that more dissociation on the LH and FSH response may be observed when positions 4, 5, 8 and/or 9 have been substituted.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This work is being continued on contract NO1-HD-0-2833.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LRF as Ovulation Inhibitors
Contract No. : NO1-HD-8-2821
Contractor : Salk Institute for Biological Studies
Money Allocated: $173,715 (FY 78); $183,276 (FY 79)

Objectives: The objective of this project is to synthesize and biologically evaluate analogs of LHRH (taking advantage of the potent antagonist, [D-p-Glu^1,D-Phe^2,D-Trp^{3,6}] LRF already developed at Salk) containing modifications at positions 1-, 2-, 3-, 6-, 7- and 10 in order to obtain very potent and long-acting antiovulatory agents.

Major Findings: Systematic modifications at positions one, two, three, five, six and seven were introduced and emphasis was put on substitutions at the one position until it was found that Ac-dehydro-L-Pro^1 was the most favored structure among those tested (Ac-dehydro-D-Pro, Ac-D- or L-thiazolidine 4-carboxylic acid, Ac-L-Phe^1 or Ac-L-Ala^1, etc...). Concentration was then placed on the two position where pCl-D-Phe, pNO_2-D-Phe, pF-D-Phe,3,4 Cl_2-D-Phe, pMe-D-Phe, pOMe-D-Phe, etc., were introduced; (see J.G. Topliss, J. Med. Chem. 20: 463, 1978: a manual method for applying the Hansch Approach to drug design). The most potent analogs in vivo (rat antiovulatory assay) were active at 5-10 µg and these were [Acylated-dehydro Pro^1, para-substituted-D-Phe^2,D-Trp^{3,6},N-Me-Leu^7] LHRH analogs. These analogs are also very potent in vitro(e.g. [Ac-dehydro Pro^1,p-Cl-D-Phe^2,D-Trp^{3,6},N-Me-Leu^7]LHRH exhibits an ICR_{50}-inhibitory concentration ratio - of 0.04/1 of antagonist to agonist in a pituitary cell culture assay and has 20 times higher affinity than LHRH in a radio-receptor assay using pituitary membrane homogenates). Peptides such as [AcΔPro^1,pNO_2-D-Phe^2,D-Trp^{3,6}]-LRF, which are more potent than the corresponding analog with pCl-D-Phe^2 in the radio-receptor and in vitro tests but not in the in vivo assay, perhaps differ in their resistance to degradation.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This project will be continued under contract NO1-HD-0-2836.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-8-2822
Contractor : University of Louisville Foundation, Inc.
Money Allocated: $118,956 (FY 78): $101,306 (FY 79)

Objectives: The objective of this project is to synthesize analogs of LHRH which contain a hydrolytically resistant non-toxic thiomethylene linkage as an amide replacement in strategic locations in order to eventually obtain very potent and long-acting antiovulatory agents.

Major Findings: The role of amide bond replacements within both LH-RH agonists and antagonists were defined. LH-RH and the parent antagonist [D-pGlu^{1},D-Phe^{2}, D-Trp^{3,6}]LH-RH, were chosen as the standards against which the pseudopeptides have been compared. Using the thiomethylene ether link as a replacement for the amide bond in positions 5-6 and 9-10 of LH-RH, substantially greater agonist activity was found compared to the 6-7 amide modification prepared last year, but neither of the agonists were as active as the parent LH-RH (2 and 5-10% as active, respectively). Among the antagonists, a 9-10 modified analog, [D-pGlu^{1},D-Phe^{2},D-Trp^{3,6},Pro ψ[CH_2S]GlyNH$_2^{9-10}$]LH-RH, had J50% of the activity of the parent antagonist in an *in vitro* assay system. Analogs modified at the 6-7 position, even with a D-amino acid at the 6-position (D-Ser ψ[CH_2S] Leu$^{6-7}$), were virtually inactive as antagonists. However, analogs with the N-terminal modifications Ac-Pro ψ[CH_2S]D-Phe$^{1-2}$,Ac-Phe ψ[CH_2S]D-Phe$^{1-2}$, or ϕCH CO-Phe ψ[CH2S]D-Phe$^{1-2}$ were active at 30 ng in the hemipituitary assay, and where a direct comparison was possible, seemed to be at least as active as their respective parent analogs. All of the potent antagonists were tested at 250 µg in an *in vivo* assay (and some at 500 µg) in the rat, but failed to show any significant anti-ovulatory activity. As a result, it has not yet been possible to determine whether the expected enhanced resistance to enzymatic degradation is an asset in these series of compounds. It is proposed that the increased lipophilicity inherent in the structures synthesized thus far might be responsible for the lack of correspondence between *in vitro* and the *in vivo* results. This possibility should be tested by newer analogs presently under preparation.

Significance to Biological Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This project will be continued under contract NO1-HD-0-2830.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthetic Chemical Facility
Contract No. : NO1-HD-8-2823
Contractor : Southwest Foundation for Research and Education
Money Allocated: $147,323 (FY 78); $156,620 (FY 79); $166,014 (FY 80)

Objective: The purpose of this project is to have this synthetic chemical facility synthesize rapidly, laboratory scale quantities of specific compounds required by the Contraceptive Development Branch for contraceptive investigation.

Major Findings: During the second year of this contract a total of twenty-two compounds were synthesized including 13 steroids and five amino acids. D,L-3,4-Dehydroproline was synthesized from pyrrole-2-carboxylic acid (317 g) using hypophosphorus acid, acetic acid and hydrogen iodide gas. The pure D and L optical isomers were obtained by preparation of the tartaric acid salts, and the salts were hydrolyzed to the free amino acids by passing through a Dowex 50W-X4 H^+ form column. N-tert-Butyloxycarbonyl-D-p-cholrophenyalanine (176 g) and N-tert-butyloxy-D-3,4-dichlorophenylalanine (12.2 g) were prepared by reaction of the racemic amino acid with trifluro acetic anhydride to give the trifluro-acetate. Optical resolution was accomplished by reaction with DFP-treated carboxypeptidase. The BOC derivatives were then prepared by treatment with di-tert-butyl-dicarbonate. Preparation of 3-methyoxy-15,16-secoestra-1,3,5(10)-trien-17-one (58 g) was accomplished in eight steps beginning with 300 grams of estrone. Such modification starting from the 3-methyl ether instead of the 3-benzyl ether was required to avoid a large scale hydrogenolysis procedure. This facility provided us with 50 g of analytically pure (99%+) Gossypol-acetic acid for testing as an oral antifertility agent for males. The crude product was recrystallized four times from a mixture of methyl ethyl ketone and acetic acid (3:1).

A number of other large batch, multi-step procedures requiring up to 10 steps were also accomplished without difficulties, i.e. a long acting progestin and others.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis of specific steroidal and nonsteroidal compounds for evaluation for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the preparation of specific chemical compounds required by the Contraceptive Development Branch and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Analysis of Data from the Study of Correlation of Cervical Mucus Changes with Serum Hormone Levels
Contract No. : NO1-HD-8-2825
Contractor : Creighton University
Money Allocated : $22,564 (FY 78), $22,093 (FY 79)

Objectives: Under a previous contract (NO1-HD-6-2823) numerous data were acquired relating menstrual cycle phenomena to the fertile period and presumptive ovulation time. The objective of the present contract is to analyze the data and to discover whether objective practical determinants of the fertile period can be derived from the data.

Major Findings: As part of the contract the investigator is completing graphic display of all of the menstrual cycles.

Significance to Biomedical Research and Program of the Institute: Utilization of the mucus cycle as a method for fertility regulation is a subject of current investigations. Research relating the mucus cycle to hormonal status is directly related to investigations supported by the Contraceptive Development Branch.

Proposed Course: Project will be completed in this fiscal year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Pharmacokinetics of Testosterone Enanthate in the Human Male
Contract Number: NO1-HD-8-2830
Contractor : Professional Staff Association of Los Angeles
Money Allocated: $66,875 (FY 78), $58,283 (FY 79)

Objectives: The proposed investigation will combine tracer kinetic studies of labeled testosterone enanthate (testosterone 7-H^3 enanthate) administered intramuscularly and intravenously on separate occasions into 7-10 normal male subjects with compartmental modeling to obtain estimates of the following pharmacokinetic parameters: plasma appearance rate; biological half-life; plasma (metabolic) clearance rate of testosterone enanthate, testosterone and estradiol; rate of conversion of testosterone enanthate to testosterone and estradiol; and the urinary excretion rate of testosterone enanthate and its metabolic products.

Major Findings: In the first 18 months of the contract period, significant progress was made in the assessment of the pharmacokinetics of testosterone enanthate (TE) in the human male. The following subjects have been studied: 9 subjects have received ^{3}H-TE intramuscularly with tracer alone and 4 subjects received tracer plus 200 mg TE. Eight subjects were treated with an intramuscular injection of 200 mg TE alone and 7 subjects were treated with 100 mg of intramuscular TE.

Methodology for separation and quantification of tracer amounts of radiolabeled TE, T and E_2 have been accomplished. Technical difficulties in the assay of unlabeled TE in the serum appear to have been overcome recently and measurement of plasma levels after TE injection are proceeding. Serum levels of T, E_2, LH and FSH after 100 and 200 mg of TE have been accomplished. Preliminary tracer analysis revealed that TE radioactivity peaked between 10 and 16 hours, fell until 50 hours and then fell more slowly until the end of the study. Plasma T radioactivity peaked slightly earlier and fell in a similar fashion to that for TE.

The addition of 200 mg TE to the intramuscular tracer dose resulted in a later peak of plasma TE followed by a long plateau (160 hours). The time course of T and its rate of disappearance were similar to TE. Preliminary analysis of the data indicates that intramuscular TE is 1) absorbed directly into the plasma as TE; or 2) converted to T and then absorbed. The prolonged half life of both T and TE suggests that these processes continue throughout the experimental period. These observations now require studies after IV tracer TE. This will allow us to determine that ½ time of disappearance of TE from plasma and plasma conversion of TE to T and its metabolites.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new male methods of contraception.

Proposed Course: Should be completed by the expiration date of the contract.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of Vaginal Contraceptives
Contract Number: NO1-HD-8-2856
Contractor : Cedars-Sinai Medical Center
Money Allocated: $124,338 (FY 78), $132,069 (FY 79), $60,741 (FY 80)

Objectives: The objective of the proposed study project is to determine the use-effectiveness and the acceptability of an intravaginal contraceptive, Encare Oval. The use-effectiveness and acceptability rates of the Encare Oval will be compared to those of a control group using the diaphragm with spermicidal gel or cream as a contraceptive method.

Major Findings: There continues to be large differences in the acceptability rates between the Encare and diaphragm users in the categories of medical and personal reasons for dropping out. The pregnancy rates remain close at 12.7 per 1200 woman months of use for Encare and 9.1 for diaphragm users. The majority of pregnancies are related to user failure and not to method failure.

The average number of months of use per Encare enrollee continues to be a major problem. The product is acceptable for those who experience infrequent intercourse. However, the vaginal warmth experienced by many users is not well tolerated by those who are, or begin to be more sexually active.

Significance to Biomedical Research and Program of the Institute:The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: Studies should be completed by the end of the contract period.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Investigation of a Polyurethane Contraceptive Vaginal Sponge Containing a Spermicidal Agent
Contract No. : NO1-HD-8-2857
Contractor : University of Southern California School of Medicine
Money Allocated: $31,081 (FY 78); $11,829 (FY 79)

Objectives: The purpose of this project is to evaluate the ability of a polyurethane contraceptive vaginal sponge containing nonyl-9 as a spermicidal agent to prevent the penetration of spermatozoa into and the migration of sperm through human cervical mucus. The effect of the sponge on the vaginal and cervical mucosa after seven days use will also be determined.

Major Findings: In the general protocol for the irritation study, 15 women were to wear a vaginal sponge for seven days, spanning midcycle. Three sponges were to be worn for two days and one for only one day. Each subject was to use non-medicated sponges in one cycle and medicated ones in the next. Fourteen subjects have completed both wear cycles, while the fifteenth has not yet used the medicated sponge. There have been no major problems using this protocol, whereas there were numerous problems in the initial phase of the study when a single sponge was left in place for 7 days. Laboratory tests were done before and after each wear cycle. The initial analyses have not shown any changes in liver function tests or serum lipid levels in conjunction with use of the medicated sponges.

In the post-coital testing, 20 women were to be tested in this phase of the study. To date, 10 women have completed the test periods. Reduced numbers of sperm were found in the mucus in all cases, as compared to the control tests. However, sperm were absent in only two women. The non-medicated sponges inhibited sperm transport in only five subjects and some sperm were present in all the subjects.

A number of other women have been screened, but were not eligible for the study because of poor baseline post-coital tests. Additional women are being screened.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: These studies should be completed by the end of this year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Primate Testing Facility
Contract No. : NO1-HD-9-2838
Contractor : Hazleton Laboratories America, Inc.
Money Allocated: $906,286 (FY 79) 3 years

Objectives: The objective of this contract program is the establishment of a primate testing facility for evaluation of the clinical potential of new contraceptive drugs, formulations and delivery systems. The present colony consists of 120 female and 31 male Cynomolgus monkeys (Macaca fascicularis). Initially they will be used to study potent analogs of LHRH and prostaglandins. The data derived from studies at this facility will determine, to a large degree, whether the institution of clinical trials is warranted.

Major Findings: Thirty females are being used in a study to determine whether sequential injections of human chorionic gonadotropin (hCG) administered during the midluteal period can simulate the hormonal conditions of early gestation by enhancing the functional integrity of the corpus luteum. The procedure appears to succeed in normal 28-day cycling animals but the injection schedule must be adjusted in long cyclers to account for the expected time of ovulation.

The daily injection of male animals with 10 or 25 µg/kg of a potent "superagonist" of LHRH for several weeks appears to have little or no effect on sperm count, motility or morphology. Circulating hormone profiles are not yet available.

Ninety females are being cycled for inclusion in the permanent colony and poor cyclers are being culled and replaced with new animals.

The colony also contains 16 breeding males.

Significance to Biomedical Research and Program of the Institute: Evaluation in subhuman primates is absolutely essential to establish the clinical potential of new contraceptive modalities before undertaking the extensive and costly safety studies required for clinical trials. Additionally, certain drugs appear to exhibit substantially different pharmacological properties in primates and in small laboratory animals thus limiting the utility of the latter. The ability of the CDB to evaluate compounds in subhuman primates is of great importance to our contract synthetic program and the stated goals of the Branch.

Proposed Course: This is expected to be a continuing contractural effort leading to the development of new contraceptive modalities and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Techniques for the Prediction and Detection of Ovulation in Humans
Contract Number: NO1-HD-9-2841
Contractor : Northwestern University Medical School
Money Allocated: $40,290 (FY 79), Approx. $70,000 (FY 80)

Objectives: The proposed study is designed to develop a simple semi-quantitative detection system for urinary pregnanediol-3-glucuronide to be used by women at home for detecting the time of ovulation. The test would employ the specific and semi-quantitative visualization on a "dip stick" of rhodamine-labeled antibody following the addition of excess rhodamine-labeled anti-body to the urine sample.

Major Findings: The feasibility of detecting ovulation in women by the use of an indicator strip that changes color in response to an increase in the concentration of pregnanediol glucuronide (PG) in urine has been investigated. Observations of an increase in the concentration of PG in urine parallel to the rise in serum progesterone after preovulatory surge of LH support the concept. Further, it has been discovered that the concentration of PG in the first urine voided in the morning has the highest concentration of PG and reflects very rapidly and reliably the increase in progesterone secretion that accompanies ovulation.

Pregnanediol glucuronide thyroglobulin has been prepared to be used in the production of antisera; antibodies have been generated with the latter antigen and purified; and purified antibody has been conjugated to tetramethylrhodamine isothiocyanate, and pregnanediol has been bound to the solid phase dip stick. Previous conjugation had been done using fluorescein, but it was found visualization (yellow color) was difficult in ordinary light. Rhodamine is more soluble in aqueous solutions, giving a better yield of conjugate, and the resulting red color is more visible in ordinary light. However, after conjugation with rhodamine, there is a decrease in binding capacity. Therefore the conditions for maximum conjugation without loss of steroid binding still have to be ascertained.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract Number: NO1-HD-9-2842
Contractor : University of California
Money Allocated: $142,863 (FY 79), $149,860 (FY 80)

Objectives: To investigate the clinical pharmacology of D-Trp^{6}-, Pro^{9}Net-LRF in normal women.

Major Findings: Recruitment of volunteers for studies involving follicular and luteal phase injections of the drug has been completed. The investigator has initiated studies to assess various routes of drug administration. Results of these studies should be forthcoming.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive drugs is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Majority of the studies should be completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract Number: NO1-HD-9-2843
Contractor : Vanderbilt University
Money Allocated: $152,975 (FY 79), $146,343 (FY 80)

Objectives: To investigate the potential of D-Trp^{6}-Pro^{9}-Net-LRF as an antifertility drug in men.

Major Findings: Volunteers have gone through the 10 week control period and some are being administered the drug on Q daily or Q every 4th day schedule. Sperm counts and hormonal endpoints are being monitored.

Significance to Biomedical Research and Program of the Institute: Development of antifertility drugs for males is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Initial projection called for completion of these studies in FY 1981. However, delays in obtaining of the IND exemption may extend these studies to FY 1982.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of a pH Barrier Contraceptive Device
Contract No. : NO1-HD-9-2845
Contractor : Research Triangle Institute
Money Allocated: $79,828 (FY 79)
Objectives: It is proposed to develop nondegradable vaginal barrier devices based on acidic hydrogel systems.

Major Findings: Films have been made from 2-hydroxyethyl methacrylate - methacrylic acid copolymer (HEMA-MMA). A large number of samples representing a complete spectrum of HEMA-MAA film compositions and morphologies have been prepared for physical characterization and biological evaluation studies. Physical characterization of HEMA-MAA copolymers have included tensile strengths and water uptake. Tensile strengths are shown to increase with increasing crosslinking concentration, increase with increasing methacrylic acid composition up to approximately 25 to 30 weight %, and increase with decreasing amount of aqueous diluent. Water uptake and swelling studies indicated that increased initial water content in the reaction mixture and high methacrylic acid content correlated with a high weight % water in the fabricated films. The extent of crosslinking shows an initial proportionality with weight % water content of the HEMA-MAA film.

Biological testing of the hydrogel films has been undertaken to determine their barrier and spermicidal efficacy. HEMA-MAA films with high MAA content exhibit much greater spermicidal activity than those of lower MAA content. Inconsistencies in spermicidal activities for test HEMA-MAA films has prompted further method development in the procedures for collecting and characterizing rabbit sperm samples. All films examined have been shown to be impermeable to live motile sperm.

Significance to Biomedical Research Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: If additional testing of this initial lead shows promise, then this project is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract Number: NO1-HD-0-2800
Contractor : Laval University
Money Allocated: $280,300 (FY 80), $265,983 (FY 81), $161,632 (FY 82)

Objectives: The objective of this contract is to investigate the potential of intranasal administration of D-Ser(TBU)^{6}-desGly-NH^{10}-LHRH-EA as an anti-ovulatory and luteolytic drug in women.

Major Findings: None. The contract has been only recently awarded.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive drugs is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Research should be completed in FY 1982.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of Vaginal Contraceptives
Contract Number: NO1-HD-0-2808
Contractor : International Fertility Research Program
Money Allocated: Approx. $220,534 (FY 80), $330,881(FY 81), $91,124 (FY 82)

Objectives: It is proposed to carry out a multi-clinic Phase III clinical trial of a newly developed non-prescription vaginal contraceptive method, the Collatex Sponge, in comparison with the diaphragm with spermicide. The incidence of adverse side effects and complaints associated with the use of the Collatex sponge compared with the diaphragm will be evaluated. Further, the rates of user compliance for the Collatex sponge will be determined. The sponge affords several significant advantages over the diaphragm. First, a single size can be used by all women; second, it is intended to be a non-prescription contraceptive; third, it is less messy than other contraceptives and can be inserted up to 24 hours in advance of coitus.

Major Findings: None, Contract not awarded yet.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract Number: NO1-HD-0-2811
Contractor : Syntex Research
Money Allocated: $778,226 (FY 80), $740,065 (FY 81), $412,372 (FY 82)

Objectives: The objectives of this contract are to assess: 1) safety of the Syntex agonist; 2) to ascertain its potential for fertility regulation in men and women.

Major Findings: None. The contract has only been recently awarded.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive drugs is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Research should be completed in FY 1982.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Luteolytic Agents
Contract No. : NO1-HD-0-2813
Contractor : Indiana University Foundation
Money Allocated: $82,074 (FY 80)

Objectives: The objective of this project is to synthesize 12-fluoro-and 12-hydroxy-prostaglandin analogs related to natural $PGF_{2\alpha}$ which might prove to be better luteolytic agents. 100 mg of (+)-15-epi-12-fluoro $PGF_{2\alpha}$ and optically pure 12-hydroxy $PGF_{2\alpha}$ will be re-synthesized for monkey studies. Smaller quantities of optically pure 12-fluoro-13, 14-dihydro $PGF_{2\alpha}$, E-$\Delta^{13,14}$-12,14-difluoro $PGF_{2\alpha}$, 12-hydroxy-13,14-dihydro $PGF_{2\alpha}$ and three 12-fluoro analogs, and their corresponding C-15 epimeric compounds will be synthesized for testing in the hamster antifertility assay, the hamster uterus and gerbil colon contraction assays.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research ad Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Postovulatory Prostanoid Antifertility Agents
Contract No. : NO1-HD-0-2814
Contractor : University of Washington
Money Allocated: $64,692 (FY 80)

Objectives: The objective of this project is to synthesize bistrifluoromethylated prostaglandins having the CF_3 groups at C-16, 17 and 18 in order to test the effect of these trifluoromethyl groups on biological activity, particularly luteolytic and smooth muscle activity.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Non-Steroidal Antifertility Agents
Contract No. : NO1-HD-0-2815
Contractor : Research Triangle Institute
Money Allocated: $93,071 (FY 80); $48,843 (FY 81)

Objectives: The goal of this contract is to synthesize simpler analogs of Zoapatanol which has been shown to induce menses and to produce a significant reduction in blood progesterone. These compounds will be tested for antifertility activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis of specific nonsteroidal compounds for evaluation for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the preparation of specific chemical compounds required by the CDB and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : New Antiprogestational Agents
Contract No. : NO1-HD-0-2816
Contractor : SRI International
Money Allocated: $125,000 (FY 80); $135,000 (FY 81)

Objectives: The purpose of this project is to synthesize the 19-nor-5α-dihydrosteroids related to 5α-dihydronortestosterone, 5α-dihydronorprogesterone, and steroids having the Ring-A inverted half-chair conformation to maximize receptor binding and test as antiprogestational agents. These compounds will be tested for antifertility activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : C-18 Functionalized Steroids
Contract No. : NO1-HD-0-2817
Contractor : State University of New York at Stony Brook
Money Allocated: $86,968 (FY 80)

Objectives: The purpose of this project is to synthesize a series of antagonists for the progestins as possible antifertility agents. By transfering the C-17 substituents to the C-18 methyl group, the compounds are hoped to be still bound to the receptors but unable to effect the subsequent biological events. These compounds will be tested for the antifertility activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Sulfur Analogs of Melatonin Derivatives
Contract No. : NO1-HD-0-2818
Contractor : Indiana University
Money Allocated: $28,877 (FY 80); $28,477 (FY 81)

Objectives: Two sulfur analogs of 6-substituted melatonin will be synthesized as an antiovulatory agent useful as a contraceptive agent.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel heterocyclic compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Functionalized Polymers as Potential Contraceptive Agents
Contract No. : NO1-HD-0-2819
Contractor : SRI International
Money Allocated: $59,645 (FY 80)

Objectives: The objective of this project is to synthesize water soluble functionalized polymers that can augment the barrier method of contraception. The hydrodynamic volume of the polymers would be such that the polymers would be non-absorbable across mucosal membranes. The polymers would consist of polymer conjugates of progestagens which would serve to inhibit spermatozoan action by interfering with plasma membrane function of the sperm.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new intravaginal contraceptives.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Antiovulatory and Postovulatory Agents
Contract No. : NO1-HD-0-2820
Contractor : Research Triangle Institute
Money Allocated: $149,060 (FY 80); $21,338 (FY 81)

Objectives: The goal of this contract is to synthesize analogs of 5α- and 5β-dihydronorethindrone which may be antiprogestational agents. These compounds will be tested for antifertility activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of New Potent Oral Antigonadotropic Agents for Male Fertility
Contract No. : NO1-HD-0-2828
Contractor : SRI International
Money Allocated: $119,048 (FY 80); $128,383 (FY 81)

Objectives: The goal of this contract is to synthesize new potent oral antigonadotropic androgenic agents for male fertility control. These compounds will be tested for the male contraceptive activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Male Contraceptive Agents: Gossypol Derivatives
Contract No. : NO1-HD-0-2829
Contractor : SISA
Money Allocated: $131,000 (FY 80)

Objectives: The objective of this project is to synthesize derivatives of gossypol and to test them for antifertility effects and for toxicity in male rats.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards the development of male contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Inhibitors of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-0-2830
Contractor : University of Louisville Foundation
Money Allocated: $91,382 (FY 80)

Objectives: The objective of this project is to synthesize LHRH analogs that contain modified peptide backbone structures by replacement of amide bonds with thiomethylene [CH_2S] and its sulfoxide [CH_2SO], alkylthiomethylene [CHRS] and its sulfoxide [$CHCH_3SO$] and aminomethylene [CH_2NH]. These amide bond replacements might provide LHRH analogs with increased half lives of circulation and increased lipophilicity while maintinaing conformational features necessary for receptor interaction.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-0-2831
Contractor : Tulane University School of Medicine
Money Allocated: $171,238 (FY 80) $183,225 (FY 81)

Objectives: The objective of this project is to prepare LHRH analogs modified in positions 1,2,3,5, and 10 to obtain more potent inhibitory activity. Fluorinated analogs of LHRH will also be examined. The analogs will be tested for in vivo and in vitro activity and receptor binding experiments will be conducted.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-0-2832
Contractor : University of Colorado Health Sciences Center
Money Allocated: $176,746 (FY 80)

Objectives: The objective of this project is to design and synthesize antagonists of LHRH modified in such a manner as to increase receptor binding and improve resistance to enzymatic degradation. The research will focus on modifications to [N-Ac-Ala^{1},D-p-Cl-Phe^{2},D-Trp^{3,6}]LHRH, one of the most potent analogs known. In vivo and in vitro assays will be performed.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-0-2833
Contractor : University of Texas at Austin
Money Allocated: $474,634 (FY 80)

Objectives: The objective of this project is to synthesize analogs of LHRH to produce antagonists with increased potency and duration of action. The most potent antagonist known to date will be modified extensively at position 1. This will be followed by a search for new flexible positions to be substituted to further increase potency. In vivo and in vitro testing will be conducted.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-0-2836
Contractor : Salk Institute
Money Allocated: $401,538 (FY 80)

Objectives: The objective of this project is to improve the potency of presently available LHRH antagonists by designing and synthesizing analogs based upon Hansch analysis as modified by Topliss. The premise is that improved potency of existing antagonists can be achieved if the predominant factors (hydrophobicity, electronic distribution or steric factors) affecting the antagonist's efficacy can be identified for loci which contain aromatic side chains. In vitro and in vivo assays will be preformed as well as receptor binding studies.

Major Findings This project has only recently begun and there are no results at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Isolation and Purification of Rat Androgen Binding Protein
Contract Number: NO1-HD-0-2845
Contractor : Population Council
Money Allocated: $106,671 (FY 80); $91,912 (FY 81); $48,460 (FY 82)

Objectives: The purpose of the proposed research is to isolate and purify androgen binding protein (ABP), to develop a radioimmunoassay for ABP, and to prepare radioimmunoassay kits for distribution to the scientific community. ABP is produced by the Sertoli cells upon stimulation by follicle stimulating hormone and its function is to transfer androgens to the tubular compartment of the testis and caput epididymis. Interference with the activity of ABP or its rate of synthesis could provide a means of selectively influencing the Sertoli cell response and, in turn, the regulation of spermatogenesis. A specific and sensitive ABP radioimmunoassay would be very useful for detecting early changes in ABP concentration in response to FSH or other hormonal treatments.

Major Findings: None as yet--new contract.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new male methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through Septemer 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Biological Testing Facility
Contract No. : NO1-HD-8-2813 continued as NO1-HD-0-2846
Money Allocated: $700,623 (FY 78); $845,501 (FY 79); $156,490 (FY 80); $839,980 (FY 80 under NO1-HD-0-2846); $1,085,506 (FY 81); $1,178,0832 (FY 82); $1,278,131 (FY 83); $1,386,882 (FY 84)

Objectives: The objective of this contract program is the establishment of a biological testing facility capable of broad spectrum evaluation of new drugs and devices and rapid exploitation of potential leads in the control of fertility. Sources of compounds for testing include contract synthetic programs supported by the CDB and numerous private, public and governmental laboratories throughout the world. The staff of the CDB reviews all compounds submitted for evaluation to determine the precise manner in which each drug will be studied.

Major Findings: The testing facility continues to evaluate new drugs, formulations and delivery systems in an efficient manner and has extended its capabilities to include radioimmunoassay of natural and synthetic steroids.

Emphasis continues to be placed on the screening of potent new analogs of LHRH. Both antagonists and agonists have been identified which exhibit biological activity with as little as a few micrograms.

A substantial number of long-acting androgens and estrus-suppressing steroids continue to be evaluated for potency and longevity of action. Several compounds have been identified which exhibit sufficient activity to warrant extended assessment.

A potent long-acting analog of norethindrone is being studied as a potential long-acting injectable contraceptive.

Data generated at the facility are entered into a computerized data analysis and storage system and are usually available to CDB staff through their inhouse terminal within 24 hours of experiment termination. Data from long term studies are entered weekly so that the progress of the study may be monitored on a regular basis.

Significance to Biomedical Research and Program of the Institute: The heart of any drug development program is the rapid identification of biological activity and exploitation of promising leads. The ability of the CDB to evaluate compounds and devices in a uniform manner is of great importance to our contract synthetic program and the stated goals of the Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Center for Population Research

The Contraceptive Evaluation Branch is responsible for a comprehensive research contracts program on the evaluation of the safety and effectiveness of methods of contraception and fertility regulation which are in use or under development. Major program components deal with (1) pathogenetic mechanisms by which serious adverse health effects of hormonal contraceptives arise (2) health risks and benefits associated with the use of hormonal contraceptives, of intrauterine devices, of male and female sterilization and of possible effects of prior induced abortion on subsequent reproduction. It is planned to continue to expand the contract program dealing with pathogenetic mechanisms and, because of increasing use, to initiate safety and effectiveness studies related to barrier contraceptives.

Steroid Contraceptives

Two studies were initiated to confirm and evaluate the presence of abnormal proteins in the serum of women taking oral contraceptives, as reported earlier in the literature. A third study looking into pathogenetic mechanisms underlying known complications of oral contraceptive use will evaluate longitudinal changes in serum lipoproteins among women using various preparations currently available.

A case-control study of myocardial infarction in young women on oral contraceptives has confirmed observations made in the United Kingdom. The risk of myocardial infarction for current pill users, relative to the risk for nonusers was estimated to be 4. For women who smoked heavily and were current pill users, relative to women who did not smoke and were not current pill users, the risk estimate was 39. Contrary to earlier analysis, past use of the pill (more than 5 years) may also increase the risk of myocardial infarction.

After an extended developmental phase, a major case-control study of breast, endometrial, and ovarian cancer in relation to use of steroid hormones was launched in cooperation, and with the financial assistance of the National Cancer Institute. While the study is just beginning, it is expected to recruit 6,000 incident cases of breast cancer, 1,500 of endometrial cancer, and 900 of ovarian cancer among women under 55 from a network of population-based U.S. cancer registries over the next two years. Exising evidence points to the possibility of a protective effect in the case of endometrial and ovarian cancer. This study will seek to confirm this important suggestion and yield a definitive analysis of breast cancer after long-term use of steroid contraception.

A large, collaborative study of the relative risk of pituitary adenoma among women on contraceptive steroids continued to make good progress. Data collection is nearly complete and it is anticipated that analysis will include 235 women with confirmed pituitary adenoma, 150 equivocal cases, and 170 women with secondary amenorrhea without evidence of pituitary tumor, and one normal control subject for each case.

Work continues on a study of the relative risk of clinical diabetes among users of oral contraceptives, with data analysis expected in 1981. It has long been recognized that use of steroid contraceptives alters carbohydrate metabolism, but the clinical significance of these biochemical changes remains unclear.

Although one study of malignant melanoma and pill use was delayed by local clearance procedures, it is now proceeding smoothly, as is a second case-control study in Australia. Analysis of data will begin in late 1981 and late 1980, respectively.

A clinical trial of the effect of folic acid administration among women with cervical dysplasia and taking oral contraceptives suggests that folic acid may halt, and possibly reverse cervical disease. Discussions are underway with the National Cancer Institute to incorporate this finding into their established program on nutritional factors and cancer. A review of the literature on folic acid, nutritional status, cervical megaloblastosis, dysplasia and neoplasia was prepared for NCI use.

Three studies of the effect of steroid contraceptives on blood pressure remain at different stages of analysis. One, conducted among a large population of Black pill users in Atlanta reports no association. While this finding is in conflict with earlier reports, it is known that pill formulations have changed since their introduction in the early 1960s and the population of pill users has become younger. Final reports from the other two studies are not yet available, but it seems likely that at least one of them will report a small association between steroid contraceptive use and hypertension, especially among heavier women. When final analyses are available, it is likely that a workshop will be organized to discuss the new results and the scientific issues they raise.

The Walnut Creek Contraceptive Drug Study closed in March 1980. A final monograph will be printed by the GPO late in 1980 and tapes will be available shortly thereafter for further analysis by qualified investigators.

Sterilization

A glycolipid antigen has been identified from guinea pig sperm and its chemical structure, made up of carbohydrate and lipid, has important implications. Similar sperm antigens apparently occur in other species, including man, and the glycolipid structure offers an important new potential mechanism for the development of atherosclerosis after vasectomy.

The cohort study of vasectomized males continued to make satisfactory progress with data collection expected to be complete in 1 to 1-1/2 years. Three additional epidemiologic studies were funded to evaluate the possible risk of accelerated atherosclerosis after vasectomy, as suggested by published monkey studies. One will re-examine an established data file of coronary angiographs in relation to vasectomy history. A second will compare vasectomy history in men with coronary symptoms or with documented heart attacks with healthy control subjects. The final study is a classic case-control study of men under 55 with first myocardial infarction in 75 hospitals.

Intrauterine Devices

Analysis of data from a large, collaborative case-control study completed this year has confirmed that IUD use increases the risk of pelvic inflammatory disease, but also shows that oral contraceptive use and the use of barrier methods protect against upper genital tract infection. The study also confirms the finding that women with an IUD in place at the beginning of their second trimester of pregnancy are at increased risk for septic fetal loss. An IUD in place at conception, but removed in the first trimester of pregnancy does not increase this risk.

The study also provides evidence that (1) IUD wearing is not responsible for the tripling of ectopic pregnancies in the U.S. since 1965, (2) IUD use does not increase the risk of abruptio placenta or placenta previa, (3) the highest risk of perforation following IUD insertion is among postpartum women who are lactating, and (4) vaginal hemorrhage serious enough to require hospitalization is not associated with IUD use.

Effects of Induced Abortion on Subsequent Pregnancies

Important findings continued to emerge from three sponsored studies. Preliminary analysis of one, expected to be completed in 1981, suggests a higher rate of spontaneous abortion and fetal death among women with prior induced abortion. These tentative findings are in general agreement with an earlier report from the Kaiser Hospitals in Northern California. A second, clinically-based study is now under analysis.

The third study indicates that women with one previous induced abortion are not at higher risk of short gestation/low birth weight, complications of labor and other problems when they carry their second pregnancy to term. A "parity" effect on outcome was seen in this study, indicating that inappropriate comparison groups could bias interpretation of the findings.

A critical variable appears to be the method of cervical dilation used. The first study will permit analysis of induced abortion performed with laminaria slow cervical dilation) against those procedures performed more rapidly with standard metal dilators.

Infertility and Contraceptive Practice

Two case-control studies were initiated to examine the relationship, if any, between past birth control practice and infertility.

Workshops and Meetings

In collaboration with the NHLBI, the CEB helped organize an epidemiology session for a conference on the influence of female hormone on vascular disease and hemostasis in Great Barrington, Massachusetts, in June 1980.

In July 1980, a two-day workshop was held on prostaglandins, thromboxanes, and side effects of oral contraceptives. Leads emerging from this meeting will be developed as part of the expansion of the contract program exploring pathogenetic mechanisms for side effects of hormonal contraceptives.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title:	Central Repository for Tissue Evaluation of Contraceptive Steroids
Contract Number:	NO1-HD-2-2729
Contractor:	Universities Associated for Research and Education in Pathology
Money Allocated:	No FY 1980 Funds

Objectives: The purpose of this project is to evaluate the pathologic effects of contraceptive steroids in long-term toxicity studies, primarily in beagles and monkeys. Tissue specimens are obtained from studies conducted by pharmaceutical companies and others, and subjected to detailed pathologic evaluation at the Armed Forces Institute of Pathology.

Major Findings: Studies have been completed on all canine specimens from the FDA-sponsored study of three investigational progestins and mestranol. The malignant tumors occurring with these steroids are no different morphologically from those reported in surveys of spontaneous canine tumors, but they occurred at younger ages and anaplastic carcinomas were more frequent. Comparative pathologic studies of spontaneous canine mammary lesions have also been completed, including evaluation of early dysplastic lesions detected in whole mount preparations. These lesions were found in the majority of bitches aged 3-4 years, but not in younger animals. Myoepithelial cell hyperplasia, a frequent component in the small palpable nodules in steroid-treated beagles, is rare in these spontaneous mammary lesions. Histopathological and ultrastructural evaluation of tissues from rhesus monkeys treated with these investigational steroids is nearing completion.

Significance to Biomedical Research and the Program of the Institute: The Food and Drug Administration requires long-term toxicity testing in beagles and monkeys for steroids proposed for contraceptive use. A number of progestins have been removed from the market because of the development of mammary nodules in beagles, but the nature of these tumors and their relevance to human cancer have been controversial. This contract provides expert, unbiased evaluation of tissues from canine and monkey toxicity studies, and comparison with human pathology, as a guide in interpreting data from these and other studies.

Proposed Course: Work on this project is to be completed this year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Secondary Amenorrhea, Pituitary Adenoma and Oral Contraceptives

Contract Number	Contractor	Money Allocated
NO1-HD-7-2834	Johns Hopkins University	$ 164,462
NO1-HD-9-2810	University of Southern California	50,159
NO1-HD-9-2811	Johns Hopkins University	33,566
NO1-HD-9-2812	University of California, San Francisco	60,559

Objectives: These contracts support a collaborative case-control study to determine relationships between secondary amenorrhea with and without pituitary adenoma, and previous use of oral contraceptives. Patients meeting specified criteria for secondary amenorrhea and/or galactorrhea, prolactin-secreting pituitary adenoma, or elevated prolactin levels, are interviewed to determine their contraceptive history. Data for comparison are obtained from matched normal controls. The data will be analyzed to determine whether oral contraceptive use previous to diagnosis was more common or of longer duration in patients in one or more of the disease categories than in the controls.

Major Findings: No findings are available as yet, but the study is progressing well. A detailed protocol, questionnaire, and manual of procedures have been developed, data collection is nearing completion, and planning for analysis of the data is underway. It is anticipated that the study will provide information on approximately 235 women with confirmed pituitary adenoma, 150 equivocal cases, 170 with secondary amenorrhea without evidence of pituitary tumor, and one normal control subject for each case.

Significance to Biomedical Research and the Program of the Institute: Pituitary adenomas are being diagnosed in increasing numbers of women with secondary amenorrhea. This may result from improved radiographic and radioimmunoassay techniques which can now identify very small tumors, or it may be that these tumors are increasing in frequency. Many of these tumors have been reported in women who have discontinued use of oral contraceptives. This study should determine whether there is a correlation between the occurrence of pituitary adenomas and previous pill use, and should distinguish a possible causal relationship from the reappearance of a tumor present before pill use but masked by the steroid medication.

Proposed Course: Data collection in this study is expected to be complete early in FY 1981 and analysis of the data will require a further six months.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Interagency Agreement Title:	Oral Contraceptive Use and the Risk of Breast, Endometrial, and Ovarian Cancer
Interagency Agreement Number:	YO1-HD-8-1037
Agency:	Center for Disease Control, Atlanta, Georgia
Money Allocated:	$4,057,713 (includes $2,500,000 from National Cancer Institute)

Objectives: To evaluate the relationship between oral contraceptive use and the occurrence of breast, endometrial, and ovarian cancer by means of a population-based case-control study of incident cases of breast, endometrial, and ovarian cancer.

Major Findings: During the developmental phase (May 1, 1978 - June 30, 1980) of this project, the Center for Disease Control (CDC) has developed and field-tested the study design, data instruments, and operational procedures for a population-based, case-control study of the relationship between oral contraceptive use and the occurrence of breast, endometrial, and ovarian cancer. Cases will be ascertained from a network of population-based cancer registries across the United States. Approximately 6,000 incident cases of breast cancer, 1,500 of endometrial cancer, and 900 of ovarian cancer that occur among women less than 55 years of age will be studied during two years of data collection. Controls will be selected via a random digit dialing procedure; approximately 6,000 controls will be studied.

Significance to Biomedical Research and Program of the Institute: This study is designed to rigorously address the question of whether oral contraceptive use affects the occurrence of breast, endometrial, and ovarian cancer.

Proposed Course: The implementation phase of the study commenced June 30, 1980. In preparation, CDC has solicited, received, and reviewed contract proposals for 1) data processing and random digit dialing; 2) case-ascertainment and interviewing. Contracts for these functions will be negotiated and made effective during July-September, 1980. Data collection will then begin, and will require 2 years. Analysis and interpretation will follow.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title:	Case-Control Study of the Relative Risk of Malignant Melanama of the Skin Among Women on Contraceptive Steroids
Contract Number:	NO1-HD-8-2803
Contractor:	University of California, Los Angeles, California
Money Allocated:	$350,234 from FY 78, FY 79, FY 80 and FY 81 Funds

Objectives: These are to estimate the relative risk of malignant melamona in women exposed to steroid contraceptives taking confounding variables of sunlight exposure and sun sensitivity into account. One hundred fifty incident cases are to be ascertained from the two population-based tumor registries in Los Angeles and the five county San Francisco Bay area. Incidence cases are preferred to prevalence cases since the effect of steroid contraceptives on survival time is not known and to avoid errors in recall and other biases in the interpretation and generalizability of results. For each case, two controls are to be selected, one of which is to be a longstanding friend, the second a neighbor. Both are to be matched for age and skin color. They will serve as partial controls for life-style, sun exposure and sun sensitivity, judged from complexion and skin color. Variables not matched in the design are to be analyzed as covariates. Both matched pair and covariate analysis are to be used.

Major Findings: None have yet been made. Progress consists of the following operational accomplishments: 1) The necessary questionnaire was developed. 2) OMB clearance of the questionnaire was obtained. 3) A Manual of Operations was developed. 4) A coding form was designed and coding instructions developed and tested. 5) Half the interviewing phase has been completed. 6) The observed number of cases was less than expected. This required extending the diagnosis date to March 1980 which offered the advantage of minimizing bias due to poor memory recall. 6) All completed interviews have been edited and coded. 7) 178 completed coding forms have been keypunched. 8) In the pathology slide review, 105 reviews have been completed. 9) The skin tone measurements have been standardized by assigning reflectance values to the perceived colors.

Significance to Biomedical Research and Program of the Institute: A major concern with safety of oral contraceptives is their potential causal association with malignant diseases. Malignant melanoma of the skin is one of several clinical entities for which such as association has been suggested but not established.

Proposed Course: Due to unavoidable delays in establishing the operational prerequisites for interviewing, and the lower than expected number of incident cases with malignant melanoma available for ascertainment, the contract period for operation and analysis was extended from December 13, 1980 to September 12, 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title:	Case-Control Study of the Relative Risk of Malignant Melanoma of the Skin Among Women on Contraceptive Steroids
Contract Number:	NO1-HD-8-2804
Contractor:	University of Sydney, Sydney, Australia
Money Allocated:	$94,014 from FY 78, FY 79, and FY 80 Funds

Objectives: These are to characterize the association between use of oral contraceptives and occcurrence of malignant melanoma, the determination of relative risk, causative and confounding factors. The most recent 300 prevalent cases in women aged 15-49 years, diagnosed since 1970 are to be ascertained from the files of the Sydney Hospital, Melanama Unit. Further, 100 incident cases are to be ascertained as they are diagnosed in Sydney Hospital during the first 18 months of this contract. For each case selected, one neighborhood and one hospital control matched by year of birth is to be ascertained.

Cases and controls are to be interviewed using a questionnaire to obtain contraceptive reporductive history and sunlight exposure. The presence of confounding factors will be sought by stratification of data according to age, parity, marital status, sunlight exposure, skin color or sun sensitivity. It is projected to be able to detect a relative risk of 1.8 with 90% certainty at a 1% significance level.

Major Findings: None have yet been made. Progress consists of the following operational accomplishments: Data collection from interviews of more than 80 new and 230 old cases with malignant melanoma, and 626 controls has been completed. The questionnaire information has been coded, punched, checked and entered into London University's computer. Preliminary tabulations and analyses are being prepared at present. It is estimated that the analysis of 1,200 interviews is needed to define the risk for malignant melanoma with any precision.

Significance to Biomedical Research and Program of the Institute: This is marked since a major concern with safety of oral contraceptives is their potential, causal association with malignant diseases.

Proposed Course: 1) The interviewing of cases and controls will be completed. 2) This operational phase of the project is to be followed by the analysis of the collected data. 3) The final report and manuscript for publication are then to be submitted. All three of these tasks are to be completed by June 30, 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Decreased Risk of Breast Disease Among Oral Contraceptive Users: Histopathologic Evaluation of Epithelial Atypia
Contract Number: NO1-HD-9-2813
Contractor: Stanford University, Stanford, California
Money Allocated: 0

Objectives: To evaluate whether the reduced risk of benign breast disease which is associated with oral contraceptive use of about two to four years duration or longer varies for different histopathologic forms of benign breast disease.

Major Findings: A suitable epidemiologic and histopathologic data base for this new project has already been established under previous contracts with NICHD and NCI. Analysis of that data base is under way. Preliminary results show an increasing risk of breast cancer in relation to oral contraceptive use as the degree of epithelial atypia increases, while for benign breast disease the opposite is seen: decreasing risk in relation to oral contraceptive use as the degree of epithelial atypia increases. However, the pattern of these associations is not clearcut and the findings are of marginal statistical significance.

Significance to Biomedical Research and Program of the Institute: One published study indicates that oral contraceptive use of about two or four years or longer decreases the risk of those histopathologic forms of fibrocystic breast disease (the most common form of benign breast disease) which are not, or are only weakly, associated with an increased risk for subsequent breast cancer. That study suggests that oral contraceptive use does not decrease, and may increase, risk for those histopathologic forms of fibrocystic breast disease which are more strongly associated with increased risk for subsequent breast cancer. This contract will provide additional data on this important issue.

Proposed Course: This project is scheduled for completion this year. The final analyses will focus on further evaluation of the relationship between oral contraceptive use and the occurrence of different histopathologic forms of benign breast disease, with detailed assessment of possible confounding or interaction from factors such as menstrual or reproductive history.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-5-2830
Contractor: Evans County Health Department, Claxton, Georgia
Money Allocated: $453,227 from FY 75 - FY 80 Funds

Objectives: This is a prospective cohort study to determine change in blood pressure before and subsequent to initiation of contraceptive steroids, in a biracial population residing in the southeastern hypertension stroke belt in southeastern Georgia. Undefined geographic pecularities in this region determine the highest mortality from hypertensive stroke for both whites and blacks in the United States. This study will answer the question whether the geographic determinants also effect an increase of hypertension associated with the use of oral contraceptives. Effects of predisposing, hereditary factors on contraceptive induced blood pressure elevation are to be investigated.

Major Findings: 22,097 patient clinic visits to seven Family Planning Clinics located in six counties of southeastern Georgia were screened to determine and enroll 1,001 black and white women meeting appropriate eligibility criteria. The follow-up rate during a two year prospective period was 84.3%. The mean blood pressure increase among oral contraceptive users, as compared to nonusers of these drugs appeared small, i.e., approximately 4 mm. mercury. However, 60 women developed blood pressure levels greater than 140/90 which automatically required they be taken off their contraceptive. These 60 hypertensive participants are being studied to identify characteristics which may make them susceptible to hypertensive effects of oral contraceptives. A two year follow-up program is available and operative to collect follow-up data on these patients. Its operation is not dependent on funds from this contract. However, it should provide documentation of the natural history of steroid-induced blood pressure elevation following cessation of the pill.

Significance to Biomedical Research and Program of the Institute: In conjunction with the findings from Atlanta (HD-5-2831) and Boston (HD-5-2832), the forthcoming findings of this Evans County Project might shed unexpected light on what once was believed the most frequent major adverse health effect of OCAs, i.e., hypertension.

Proposed Course: The operational phase of the project is completed. Analysis of the completed data has begun. The final results will be published.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effect of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-5-2831
Contractor: Emory University
Money Allocated: $740,000 (FY 1975 through FY 1978 funds)

Objectives: 1) Estimation of prevalence, incidence and magnitude of blood pressure elevation in a cohort of black contraceptive users in Atlanta, Georgia. 2) Assessment of variables, determining and confounding this association.

Major Findings: No significant ($p<0.05$) association between oral contraceptive use and blood pressure of 20,639 southeastern women, examined between September 1975 and August 1978, was found. This conclusion is based on four categories of data: (1) The prevalence and incidence of hypertension in oral contraceptive users were not higher than in non-users. Over 1,000 women with elevated blood pressure were identified equally frequent in users than in non-users. (2) The family history of hypertension does not differ between hypertensive contraceptive users and women with essential hypertension. (3) The clinical and laboratory features and medical histories of hypertension in oral contraceptive users do not differ from essential hypertension without contraceptive therapy. (4) The spontaneous and therapeutic reversibility of hypertension in contraceptive users do not differ from essential hypertension in the absence of contraceptive therapy.

Significance to Biomedical Research and the Program of the Institute: These findings are contrary to all previous reports, which gave rise to John H. Laragh's published opinion: "By conservative standards about 5% of women using oral contraceptives develop overt hypertension." This number of contraceptive induced hypertension, in about half a million women in the United States, would be larger than the combined frequencies of all other diseases attributed to adverse effects of OCAs. Also, the above findings or interpretations from Emory University, not only conflict with all previous and all published reports, they also differ from the forthcoming results of the other two current projects: -HD-75-2830 and -HD-75-2832. While in these latter two projects, at least a minimal hypertensive effect of OCA-use was observed, in this largest study, ever done on this problem, such an effect could not be demonstrated.

Proposed Course: Although the project is completed, its results would be of very limited value without answering the question as to the confounding variables determining the discrepancies of past and forthcoming findings. An analysis of the pooled data from the three current projects and that of Walnut Creek is under consideration for answering this question. Without such an analysis, the question could not be answered, as to whether or not oral contraceptives causue high blood pressure.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-75-2832
Contractor: Harvard Medical School, Boston, Massachusetts
Money Allocated: $507,462 from FY 1975-Fy 1979 Funds

Objectives: In 1973 a total community survey on all 5,000 white females, ages 16-49 years, residing in East Boston was conducted. In this survey blood pressure, contraceptive use and demographic characteristics were ascertained. This provides the basis for the objective of this project: Determine incidence of blood pressure changes associated with continued, discontinued or initiated contraceptive therapy since 1973.

Major Findings: According to the variable of contraceptive use the cohort comprises 4 subcohorts: (a) 1973 No/1976 No = continuous OCA-non-user, (b) 1973 Yes/1976 Yes = continuous OCA-users, (c) 1973 Yes/1976 No = discontinuing OCA-users, (d) 1973 No/1976 Yes = OCA-use initiators. The preliminary, partial analysis restricted itself to relating the two variables of changing contraceptive use to changes in blood pressure. This revealed no marked hypertensive drug effect, whether such an effect was minimal or absent could not yet be determined. This uncertainty results from a third variable: continuous non-OCA-users were 10-15 years older and also heavier than the cohort of new OCA-users. The other two subcohorts were intermediate. This age difference between the subcohorts, defined by OCA-use status might account for as much or more than 5 mm H_g difference in blood pressure and precludes simple interpretation of the variable, blood pressure in terms of the variable contraceptive use Furthermore OCA-use may be associated with blood pressure elevation in heavier females, more than 25-30 years old, but not in adolescent teenagers, less than 20 years old.

The still forthcoming final analysis suggests a slight or minimal hypertensive effect of OCAs; possibly contrary to the Emory findings in Atlanta, Georgia, on blacks, where not even a minimal effect was found.

Significance to Biomedical Research and the Program of the Institute: Presently, this is uncertain because the forthcoming estimates as to the hypertensive effects of oral contraceptives seem conflicting and contradictory to all previously published ones. This conflicting uncertainty may be resolved by testing the following hypothesis: The variance of hypertensive effects observed in different projects is due to the variance of age and the narrow age range of contraceptive users examined in each of the current projects and the past Walnut Creek project.

Proposed Course: An analysis of the pooled data from the three current projects and that of Walnut Creek is under consideration. Without such an analysis the question could not be answered whether or not oral contraceptives cause high blood pressure.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Case-Control Study of Myocardial Infarction in Young Women with Special Reference to the Role of Oral Contraceptives
Contract Number: NO1-HD-6-2849
Contractor: Boston University, Boston, Massachusetts
Money Allocated: $12,303

Objectives: To evaluate the relationship between oral contraceptive (OC) use and the occurrence of non-fatal myocardial infarction (MI) among premenopausal women. Special emphasis is being placed on evaluation of possible interactions between OC use and other MI risk factors such as cigarette smoking, hypertension, blood lipids, etc.

Major Findings: Two hundred thirty-four premenopausal women with first MI have been compared to 1,742 hospitalized controls. (Lancet 1:743-747, 1979). The overall risk of MI for current OC users (within the month preceeding hospitalization), relative to the risk for non-users, was estimated to be 4 (95% CI: 2.5-6.3). For women who smoked heavily but were not current OC users, relative to women who did not smoke and were also not current OC users, the risk estimate was 7 (95% CI:5/12). For women who both smoked heavily and were current OC users, relative to women who did not smoke and were not current OC users, the risk estimate was 39 (95% CI:22-70). In addition to this effect of current OC use, recent analyses indicate that past OC use of long duration (more than 5 years) may increase the risk of MI.

Significance to Biomedical Research and Program of the Institute: The relationship between OC use and the occurrence of MI is an issue of substantial public health importance. The project is providing data which are needed to clarify this relationship.

Proposed Course: Collections of data from new cases and controls ceased in June, 1979. All available cases and an appropriate sample of controls are being re-interviewed at home following hospital discharge. A blood specimen is being obtained for cholesterol, triglyceride, high density lipoprotein, and ABO blood type determinations. A type-A, type-B personality profile will be obtained. These data will be incorporated into the existing data file (demographic characteristics, medical, surgical, reproductive, and birth control history, smoking, alcohol use, etc.), and the relationship between OC use and the occurrence of MI is being re-analyzed to evaluate the relationships between all of the risk factors for which data have been obtained. The project is scheduled to terminate in December, 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Oral Contraceptives on Folic Acid Metabolism
Contract Number: NO1-HD-74-2862
Contractor: University of Alabama School of Medicine
Money Allocated: $98,797 (1978); $79,115 (1979);$19,195 (1980)

Objectives: These objectives are based upon striking findings of unknown medical significance by Whitehead, Reyner and Lindenbaum. Cervical smears in 19% of contraceptive steroid users revealed "undefined" morphological abnormalities. They were similar to those (a) accompanying erythropoetic megaloblastosis found in Vitamin B_{12} and folate deficiency and (b) of the premalignant lesion: cervical dysplasia. In spite of absence of systemic folate deficiency, administration of monoglutamic folic acid (10 mg daily for three weeks) reversed or cured all megalocytotic abnormalities. It has never been investigated whether these morphologic abnormalities in the absence of folate therapy may actually progress towards cervical dysplasia. The objectives of this project were to assess the efficacy of folate therapy for reversing cervical dysplasia in users of oral contraceptives (clinical trial).

Major Findings: The following scoring system was applied for numerical quantitation and analysis of cytologic and histologic cervical findings: 1= Normoplasia or negative; 2=Mild dysplasia; 3=Moderate dysplasia; 4=Severe dysplasia; 5=Carcinoma in situ. The pooled mean pretreatment scores were 2.34 ± 0.79 and not statistically different among or between the subjects subsequently treated with placebo or folate, i.e., 2.32 ± 0.70 for the placebo group and 2.40 ± 0.78 for the folate group. Treatment with folate was associated with a consistent downward trend, i.e., improvement in cytology scores. Using the "t" test for paired data, the final cytology score for the folate treated oral contraceptive users was significantly better than the initial scores ($p<0.05$). In contrast, the mean cytology score over the short three months treatment and observation period, remained essentially unchanged in oral contraceptive users treated with placebo. By necessity, the results have to be predominantly based on comparisons of cytological findings or scores before and after treatment. For the more meaningful histological diagnoses after treatment, no baseline comparisons before treatment are available. The necessary biopsies for histologic examinations, of course, might have removed the lesions and prevented their further investigation. However, such examinations yielded five cases of carcinoma in situ in the placebo treated group. None of the dysplastic lesions among the oral contraceptive users treated with folate progressed to this carcinomatous stage.

Significance to Biomedical Research and the Program of the Institute: Potentially the above findings may provide the basis for the first specific nutritional therapy of a premalignant, cancerous lesion, via a nutritional intervention in its pathogenesis or carcinogenesis; i.e., correction of abnormal nucleic acid synthesis resulting from impaired folate dependent nucleotide systhesis and leading to defective nuclear maturation.

Proposed Course: The project is completed

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Relative Risk of Diabetes in Users of Steroid Contraceptives
Contract Number: NO1-HD-8-2818
Contractor: Emory University
Money Allocated: $187,000

Objectives: The purpose of this study is to determine whether steroid contraceptive use, which is known to result in changes in glucose tolerance, is associated with an increased risk of overt clinical diabetes mellitus. The contract supports a classical case-control study comparing current and past use of oral contraceptives in 150 women with newly diagnosed diabetes mellitus and 450 control women matched for age, race and socioeconomic status. Data on known or suspected risk factors for diabetes are also being obtained.

Major Findings: This study is progressing well but findings are not yet available. Data collection has been completed for 151 eligible cases and for over 300 eligible controls. Interviews with the remaining controls are expected to be completed early in FY 1981. Planning for analysis of the data is in progress.

Significance to Biomedical Research and the Program of the Institute: Oral contraceptives are now used by millions of American women. Effects of the contraceptive steroids on glucose tolerance have been reported since the early 1960s, and it has been postulated that the steroid-induced changes in carbohydrate metabolism may, with continued exposure, lead to frank clinical diabetes. No evidence on this point has been obtained from general prospective studies of OC users and no retrospective studies of the risk of diabetes have been reported. This contract is expected to answer the question of the clinical significance of changes in glucose tolerance induced by oral contraceptive use and determine whether long-term users are at increased risk of developing diabetes.

Proposed Course: Data collection for this study is expected to be completed early next year and analysis of the data will require a further six months.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Contraceptive Drug Study
Contract Number: NO1-HD-3-2710
Contractor : Kaiser Foundation Research Institute, Walnut Creek, California
Money Allocated: $28,586 (FY80)

Objectives: This prospective longitudinal study of 18,000 women who are part of a prepaid medical plan in Walnut Creek, California is concerned with the identification of hazards associated with the use of contraceptive steroids.

Major Findings: This project has produced numerous findings, which are relevant to the determination of the safety of contraceptive steroids. These include an increased relative risk of thromboembolism, stroke and subarachnoid hemorrhage and a possible slight increase in carcinoma in situ of the cervix. There is also the suggestion of a protective effect from cancer of the ovary and endometrium. Other findings of interest include an effect of oral contraceptives on certain lipoprotein fractions, glucose tolerance, and various coagulation factors. An interim report on findings has been prepared by the investigators and will be published late in 1980.

Significance to Biomedical Research and the Program of the Institute: The determination of the adverse (and beneficial) long-term health effects associated with the use of oral contraceptives is critical to the program of the Center for Population Research and the NICHD. This is one of the major efforts related to this goal.

Proposed Course: The contract has been terminated.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Long Term Effects on Mothers and Offspring of Diethylstilbestrol Treatment During Pregnancy
Contract Number: NO1-HD-8-2829
Contractor : University of Chicago
Money Allocated: $208,280 (FY 80)

Objectives: This project is to determine the effects of prenatal exposure to diethylstilbestrol (DES) on the health of male and female offspring, especially on reproduction and the risk of cancer, and also to determine whether there is a risk of cancer of the breast and other organs associated with the use of DES by the mothers.

Major Findings: Male offspring exposed in utero to DES revealed a greater proportion with minor abnormalities of the external genitalia including epidymidal cysts and also low sperm counts and decreased sperm mobility. Among female offspring no case of clear-cell adenocarcinoma of the vagina or cervix has been identified. A high proportion reveal vaginal and cervical adenosis and other related findings typical for DES in utero exposure. Extended follow-up suggests the possibility of increased primary infertility among DES-exposed daughters and a higher rate of unfavorable outcome among those becoming pregnant. Among the mothers exposed to DES while pregnant a greater risk of cancer of the breast and of the ovary and cervix was reported after the first review of the cohort which was of borderline significance.

Significance to Biomedical Research and the Program of the Institute: This is a unique study based upon a controlled clinical trial to evaluate the effectiveness of DES in improving pregnancy outcome which was conducted at the Lying-In Hospital in Chicago during the early 1950s. The determination of adverse effects of prenatal hormonal exposure on the reproductive system of female and male offspring is of critical importance to the program of this Institute.

Proposed Course: Reevaluation of the female and male offspring is concerned with the determination as to the specific effect of DES exposure on the risk of infertility in the male as well as infertility or subfertility in the female. In addition, other adverse health effects including cancer of the urogenital tract are evaluated. Further follow-up of the mothers is aimed at resolving the question as to whether there is indeed an increase risk of cancer of the breast and reproductive organs associated with DES exposure in this cohort. It is planned to conduct an external review of this contract early in 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Research on the Presence of Abnormal Proteins in the Serum of Users of Oral Contraceptives
Contract Number: NO1-HD-0-2823
Contractor: Emory University, Atlanta, Georgia
Money Allocated: $214,706 from FY 1980 Funds

Objectives: These are to measure the levels of a serum protein fraction, precipitable by 25% saturation with ammonium sulfate, in the serum of healthy women who have never used oral contraceptives, healthy women who are currently using them, healthy women who used them but have discontinued their use, and in women who have had thrombotic episodes whether taking oral contraceptives or not. This protein fraction has been reported by Beaumont et al. to be elevated in a significant number of healthy users and ex-users of oral contraceptives and to be markedly elevated in most women who developed serious thrombotic complications while using oral contraceptives. Research under this contract will seek confirmation of the work by Beaumont et al. The approach will also be extended to include a longitudinal study of women before and after initiation of oral contraception. The effect of race on this protein fraction and its response to oral contraceptives will also be investigated.

Major Findings: The project was initiated June 30, 1980. The laboratory procedures are now being established.

Significance to Biomedical Research and the Program of the Institute: This research should help to elucidate the pathogenetic mechanism involved in thrombosis related to the use of oral contraceptives and may furnish a method useful in identifying women who are at special risk of thrombosis while using oral contraception.

Proposed Course: It is anticipated that measurements on 50 users, 50 ex-users, 50 never users, 100 new users before and after initiation of use, and about 50 thrombotic patients will be completed by December 31, 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Research on the Presence of Abnormal Proteins in the Serum of Users of Oral Contraceptives
Contract Number: NO1-HD-0-2824
Contractor: Wayne State University, Detroit, Michigan
Money Allocated: $93,481 from FY 1980 Funds

Objectives: One objective is to investigate the level of a protein fraction, precipitable by 25% saturation with ammonium sulfate, in the serum from women who are currently using oral contraceptives, those who used them in the past, and those who have never used them. In addition serum from patients who have experienced thrombosis while taking oral contraceptives or not will be examined for the level of this protein fraction. These studies are being done in order to seek confirmation of the results of Beaumont et al. who have reported the presence of this material in many users and past users of oral contraceptives; but not in women who have never used oral contraceptives; high levels were reported in the serum of almost all patients who developed thrombosis while taking oral contraceptives.

Major Findings: The project was initiated on June 1, 1980. Work is proceeding on the establishment of the laboratory procedures and the recruitment of subjects.

Significance to Biomedical Research and the Program of the Institute: This research should help to elucidate the pathogenetic mechanism involved in thrombosis related to the use of oral contraceptives and may furnish a method useful in identifying women who are at special risk of thrombosis while using oral contraception.

Proposed Course: It is anticipated that measurements on 100 users, 50 ex-users, 100 never users and a group of thrombotic patients will be completed by November 30, 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Vasectomy on Testis, Epididymis and Vas
Contract Number: NO1-HD-2-2770
Contractor: University of Virginia School of Medicine
Money Allocated: No FY 1980 Funds

Objectives: This contract supported animal studies of the effects of vasectomy on intraluminal pressure and electrolyte concentrations in the testis and epididymis and the permeability of the blood-testis barrier and epididymal wall. Micropuncture techniques were used to obtain fluid samples and pressure measurements. Light and electron microscopic studies were correlated with studies of luminal contents to evaluate absorptive and secretory activity.

Major Findings: The effects of vasectomy on the blood-testis and blood-epididymal barriers to water, inulin, and urea were examined in hamsters. Subtle changes in the function of these barriers were demonstrated at 4 months after vasectomy (increased entry of water and urea into the seminiferous tubules and of water and inulin into the epididymis, and decreased entry of urea into the epididymis). Morphologically, patchy areas of degeneration were seen in the seminiferous tubules of hamsters five months after vasectomy. Some tubules had few or no germ cells, in others there were spermatocytes but no spermatids, some had all stages except late spermatids, and in others spermatogenesis was esentially normal. Organelles in the Sertoli cells of the altered tubules appeared essentially normal, and there were no changes which could be correlated with altered physiological function.

Significance to Biomedical Research and the Program of the Institute: This project has provided new information on the physiology of the male reproductive tract and alterations resulting from vasectomy. Physiological modifications not repairing themselves after vas reanastomosis may be a significant factor in persistent infertility after vasectomy reversal.

Proposed Course: Work under this contract has been completed.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunopathologic Consequences of Vasectomy
Contract Number: NO1-HD-3-2717
Contractor: University of New Mexico
Money Allocated: $6,000

Objectives: This contract has supported studies of immunopathologic effects of vasectomy in experimental animals and man. In vasectomized men, the investigations have been directed to defining the class and antigenic specificity of circulating antibodies to sperm antigens, the development of cell-mediated immunity, and the detection of circulating immune complexes. In vasectomized animals and animals with experimental allergic orchitis (EAO), pathological changes in reproductive and other organs have been evaluated and efforts have been made to define the roles of specific sperm antigens, T and B cells, and tolerance or the lack thereof, in the development of pathology.

Major Findings: During the past year, an autoantigen of glycolipid structure has been identified and partially purified from guinea pig sperm. This appears to be a small glycolipid with molecular weight of about 4-6000 daltons. It is probably a galacto-sphingolipid with 2-3 sugar residues and a galactose as the terminal residue. It was first prepared from guinea pig sperm and larger amounts have since been prepared from testicular homogenates.

Significance to Biomedical Research and the Program of the Institute: The glycolipid nature of this antigen has important implications and may offer a unique pathogenetic mechanism for the development of atherosclerosis in vasectomized individuals. By analogy with other glycolipid antigens such as those of nervous tissue, the sugar groups probably provide the antigenic determinants and the hydrophobic fatty acid portion of the molecule may, in the natural state, be inserted in the lipid bilayer of sperm surface membranes. After vasectomy, the circulating glycolipid antigen may be readily inserted into the plasma membrane of vascular endothelial cells, rendering them targets for injury by antibody and complement and initiating formation of atherosclerotic plaques.

Proposed Course: Work under this contract is expected to be completed this year. Further work on the glycolipid antigen will be supported by a research project grant.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Long-Term Effects of Vasectomy in the Rhesus Monkey
Contract Number: NO1-HD-4-2866
Contractor: Oregon Regional Primate Research Center
Money Allocated: No FY 1980 Funds

Objectives: The purpose of this contract was to evaluate the effects of vasectomy in rhesus monkeys which were vasectomized up to 14 years ago. Parameters studied included general health status, hormone levels, humoral and cell mediated immune responses to sperm antigens, autoantibodies to non-genital antigens, and complete pathologic and immunopathologic assessment at autopsy.

Major Findings: Studies have been conducted on 39 monkeys vasectomized in 1964-74, 22 age-matched controls, and 21 monkeys with special complex histories. All of the vasectomized animals developed circulating antibodies to sperm antigens and 50% retained measurable antibodies for extended periods of time. Data on cell-mediated immune responses are equivocal, as are results from other studies, at least in part because purified sperm antigens are not available for use as reagents. Clinical laboratory data and hormone levels have been reassuringly negative, but there have been significant pathological findings in two areas. First, the monkeys in this study, which were maintained on cholesterol-free, low-fat monkey chow, demonstrated a marked increase in atherosclerosis as compared to controls. This extends previous findings of exacerbation of atherosclerosis in cynomolgus monkeys fed a high cholesterol diet, generalizing the results to another species of monkeys and to animals fed a more normal diet. Significant pathological effects have also been demonstrated in the testis of vasectomized monkeys. Orchitis, aspermatogenesis, or both, similar to that seen in experimental allergic orchitis, was seen in almost all of the vasectomized animals as compared to one quarter of the controls. Deposits of immunoglobulin and complement were demonstrable in the ductuli efferentes and epididymis in most of the vasectomized animals, while similar but less extensive deposits were seen in less than half of the control monkeys. These lesions are apparently immunologic in origin.

Significance to Biomedical Research and the Program of the Institute: This project has offered a unique opportunity to evaluate the long-term effects of vasectomy in a subhuman primate. Most of the laboratory findings, except the consistent demonstration of antisperm antibodies, have been negative. The finding of increased atherosclerosis in vasectomized monkeys is unquestionable and has resulted in the initiation of studies to determine whether or not the relationship applies in man. Further studies will be needed to determine whether the testicular pathology is applicable to other species.

Proposed Course: Work under this contract has been completed.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunologic Studies of Vasectomy
Contract Number: NO1-HD-5-2842
Contractor: University of Connecticut Health Center
Money Allocated: $89,751

Objectives: This contract supports immunopathologic investigations of the effects of vasectomy, particularly the development of immune responses to sperm antigens and the incidence and course of immune complex orchitis, in laboratory animals of several species and strains.

Major Findings: Investigations of vasectomized rabbits are nearing completion. In specific-pathogen-free and standard rabbits, immune deposits are seen in the testes in about two-thirds of the vasectomized animals, and in the kidneys in about 7%. Pathologic alterations in the testes (fibrosis, infiltration of mononuclear cells, degeneration of the seminiferous epithelium) have been demonstrated in 18 of 45 vasectomized animals, and clear-cut signs of glomerulonephritis are seen in two of these rabbits.

In rats, this investigator has previously demonstrated differences between various genetic strains in the development of antisperm antibodies after vasectomy. Pathologic studies of testis and epididymis have now been conducted in vasectomized and control Brown Norway rats, a responder strain. Deposits of immunoglobulin and complement occur almost universally in the epididymis in rats of this strain, whether vasectomized or not. Testicular deposits also occur commonly, but are considerably more frequent and extensive after vasectomy. Pathologic evaluation of the testes is in progress. Atrophy, degeneration of the seminiferous epithelium, fibrosis, and mononuclear cell infiltration have thus far been detected in tissue from 23 of 25 vasectomized Brown Norway rats.

In mice, testis and epididymis from 30 vasectomized and control animals of several different strains have been evaluated. All specimens were negative until recently when heavy IgG deposits of typical distribution were seen in the testis of one vasectomized mouse and light deposits in a second mouse of the same strain. Sham operated animals of this strain were negative. Additional animals are being evaluated.

Significance to Biomedical Research and the Program of the Institute: Immune complex orchitis was first reported by this contractor in vasectomized rabbits. This finding has now been confirmed by other investigators and other types of post-vasectomy orchitis, involving lymphocytic invasion but not immune complex deposition, have been identified in guinea pigs and monkeys at long intervals after vasectomy. Studies under this contract will help define the range of pathology and some features of the pathogenetic mechanisms involved in various species of laboratory animals.

Proposed Course: Work under this contract is expected to be completed this year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Health Status of American Men

Contract Number	Contractor	Money Allocated
NO1-HD-6-2807	University of California at Los Angeles	$415,000
NO1-HD-7-2801	Mayo Clinic	210,045
NO1-HD-7-2802	University of Southern California	692,930
NO1-HD-7-2803	University of Minnesota	472,610

Objectives: The purpose of this collaborative study is to identify long-term health hazards which may be associated with vasectomy as a contraceptive procedure. The current health status and medical histories of previously vasectomized men are to be compared with those of non-vasectomized control groups. The contracts with the Mayo Clinic, USC and the University of Minnesota provide for data collection according to a common protocol, and the contract with UCLA provides for project coordination and data analysis.

Major Findings: In the first phase of this study, a detailed protocol and manual of procedures was developed and subjects for the study were identified. The study is now in the data collection phase and information has been obtained and processed on over 16,000 subjects. Plans for analysis are well under way but analyses of possible differences between cases and controls have been deferred pending completion of data collection in order to avoid any possibility of introducing bias into the collection of the remaining data.

Significance to Biomedical Research and the Program of the Institute: Vasectomy is an increasingly popular method of contraception which has generally been considered to have no adverse effects. However, it is known that a substantial proportion of vasectomized individuals develop circulating antibodies to spermatozoa and that, in rabbits, guinea pigs, and monkeys, this immune response can result in disease in the testis and sometimes kidney. Furthermore, in monkeys there is now strong evidence that vasectomy leads to exacerbation of atherosclerosis, presumably through immunologic injury to arterial walls. This study will be able to identify differences in incidence of common and also quite rare diseases in vasectomized men as compared to controls, or to demonstrate that there is no increase in risk within certain defined limits of probability.

Proposed Course: Data collection is expected to be completed in another 1-1½ years, and final analyses of the data will require a further 6 months.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title:	Effect of Bilateral Vasectomy on the Progression and Regression of Atherosclerosis in Macaca fascicularis
Contract Number:	NO1-HD-8-2827
Contractor:	Oregon Regional Primate Research Center and Bowman Gray School of Medicine
Money Allocated:	$125,144

Objectives: The purpose of this contract is to pursue preliminary findings which indicate that vasectomy may significantly increase the severity of atherosclerosis in monkeys. The effects of vasectomy on atherogenesis will be studied in normocholesterolemic and hypercholesterolemic monkeys fed a diet comparable in cholesterol content to that of the average American man. The possible therapeutic role(s) of a more prudent diet and/or vas reanastomosis on regression of established atherosclerosis will also be evaluated.

Major Findings: This study is progressing well but no findings are yet available. Sixty cynomolgous monkeys are under study at Bowman Gray and 75 at Oregon. The animals have been acclimated to the experimental diet for 6 months, and vasectomies have been performed in half of those at Bowman Gray and all of those at Oregon. The monkeys are now being followed with periodic determinations of blood lipids, blood pressure, sperm antibodies, and other parameters. At 18 months after surgery, the animals at Bowman Gray and one group of those at Oregon will be sacrificed for evaluation of the extent of atherosclerosis, and regression studies will be initiated in the remaining animals at Oregon.

Significance to Biomedical Research and the Program of the Institute: These contractors previously demonstrated that vasectomy markedly increased the extent of atherosclerosis in a small group of cynomolgus monkeys fed a high cholesterol diet and more recently they have demonstrated significantly increased atherosclerosis in rhesus monkeys maintained for 9-14 years after vasectomy on a diet free of cholesterol. If these findings are applicable to man, vasectomy, an increasingly popular method of permanent contraception, could lead to a significant increase in cerebrovascular and cardiovascular disease. This contract is designed to validate and extend the preliminary monkey data and to determine the possible therapeutic role of vas reanastomosis and/or restriction of dietary lipids.

Proposed Course: The animals in this study will be maintained on the experimental diet for 18 months. At that time, the monkeys at Bowman Gray will be sacrificed and all major arteries evaluated for amount of atherosclerosis. Fifteen of the monkeys at Oregon will also be sacrificed at 18 months after surgery and similarly evaluated. The remaining monkeys at Oregon will be used in studies to determine the value of vas reanastomosis and/or dietary restriction in regression of established atherosclerosis.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Epidemiologic/Clinical Studies of Vasectomy and Atherosclerosis
Contract Number: NO1-HD-0-2806
Contractor: Medical College of Wisconsin
Money Allocated: $86,000

Objectives: The purpose of this study is to determine whether there is a relationship between vasectomy and the extent of coronary artery atherosclerosis in man. Cardiovascular status in the 11,000 members of the Milwaukee Cardiovascular Data Registry has previously been evaluated by coronary angiography. The men are now being queried as to vasectomy history and the combined data will then be analyzed to determine the relationship between vasectomy (and duration of exposure to the effects of vasectomy) and the extent of coronary artery occlusion in various age groups. Data on a number of established cardiovascular risk factors are also available for analysis.

Major Findings: This study was initiated in February 1980 and no findings can be expected at this time. Data collection is on schedule and computer programming is in process to combine the new data with the existing data file.

Significance to Biomedical Research and the Program of the Institute: Data from two NICHD contract studies have now demonstrated that vasectomy results in marked exacerbation of atherosclerosis in two species of monkeys. The possibility has thus been raised that vasectomy, an increasingly popular method of permanent contraception, may lead to a significant increase in risk of heart and cerebrovascular disease in man. This contract should contribute important information on the effect of vasectomy on the development of atherosclerotic lesions in human coronary arteries at an early stage, before they may become evident in increased rates of clinical disease.

Proposed Course: Data collection and analysis in this project are expected to require another year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Epidemiological Study of Vasectomy and Coronary Heart Disease
Contract Number: NO1-HD-0-2809
Contractor: Battelle Memorial Institute
Money Allocated: $247,229

Objectives: The purpose of this project is to determine the relationship between vasectomy and the subsequent development of coronary heart disease. The study involves men enrolled in the University of Washington Exercise Testing Registry who have had angina pectoris, myocardial infarction or resuscitated cardiac arrest, and control subjects who have no indication of coronary heart disease. Vasectomy history will be obtained by questionnaire. Data on the prevalence of vasectomy and duration of exposure will be compared in the two groups. Data will also be analyzed to take into consideration other known and suspected risk factors for cardiovascular disease.

Major Findings: This study was initiated in June 1980 and no significant findings can be expected at this time.

Significance to Biomedical Research and the Program of the Institute: Data from two NICHD contract studies have now demonstrated that vasectomy results in marked exacerbation of atherosclerosis in two species of monkeys. The possibility has thus been raised that vasectomy, an increasingly popular method of permanent contraception, may lead to a significant increase in risk of atherosclerotic disease in man. This contract is expected to provide important information on the role of vasectomy as a possible risk factor for coronary heart disease in man.

Proposed Course: Data collection and analyses for this project are expected to require two years.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Study of Myocardial Infarction in Relation to Vasectomy
Contract Number: NO1-HD-0-2810
Contractor: Boston University, Drug Epidemiology Unit
Money Allocated: $286,600

Objectives: The purpose of this contract is to determine the relative risk of myocardial infarction in vasectomized as compared to non-vasectomized men. A classical case-control study will be conducted in some 75 hospitals in New England and New York State. Data will be obtained from male patients under age 55 admitted for first myocardial infarction and from matched hospital control subjects. Data to be obtained include vasectomy history and history of other known or suspected risk factors for cardiovascular disease. It is estimated that some 1650 cases and 3-4 controls per case will be enlisted over a three year period of data collection.

Major Findings: This study was initiated in April 1980 and no findings can be expected at this time. Collaborative arrangements are being finalized with the cooperating hospitals and data collection has been initiated.

Significance to Biomedical Research and the Program of the Institute: Data from two NICHD contract studies have now demonstrated that vasectomy results in marked exacerbation of atherosclerosis in two species of monkeys. The possibility has thus been raised that vasectomy, an increasingly popular method of permanent contraception, may lead to a significant increase in risk of atherosclerotic disease in man. This contract is expected to provide important information on the role of vasectomy as a possible risk factor for myocardial infarction in man.

Proposed Course: This project is expected to require three years for data collection, with a further six months to complete the analyses.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title:	Survey of Women's Health and Birth Control Methods	
	NO1-5-2852	$104,958
	NO1-7-2811	0
	NO1-6-2806	0
	NO1-7-2807	0
	NO1-6-2804	0
	NO1-7-2808	5,309
	NO1-7-2809	0
	NO1-7-2812	0
	NO1-6-2805	10,855
	NO1-7-2810	0
Contractors:	Biostatistics Center of George Washington University and nine clinical centers	
Money Allocated:	$215,419	

Objectives: These contractors have collaborated to conduct a multi-center case-control study of the relationship between IUD use and the occurrence of seven serious gynecological and obstetrical disorders (uterine performation, pelvic inflammatory disease, septic and non-septic fetal loss, vaginal hemorrhage, ectopic pregnancy, abruptio placenta, and placenta previa).

Major Findings: Using a common protocol and set of data forms, data has been gathered in 9 collaborating clinical centers concerning nearly 9,000 cases (all diagnostic categories) and 10,000 controls. These data have been analyzed and reports have been prepared and submitted for publication. These reports: 1) confirm that IUD use increases the risk of pelvic inflammatory disease, but also show that oral contraceptive use and use of barrier methods (diaphragm, condom) decrease risk; 2) confirm that women with an IUD in place at the beginning of the second trimester of pregnancy are at increased risk of septic fetal loss, and show that this increased risk is not present for women with an IUD in place at conception but removed in the first trimester of pregnancy 3) provide evidence that IUD use is not responsible for the tripling of ectopic pregnancies in the United States during the years 1965-1977; 4) provide evidence the IUD use does not in general increase the risk of vaginal hemorrhage serious enough to lead to hospitalization; 5) show that the risk of uterine perforation following IUD insertion is greatest for post-partum insertion in women who are lactating; 6) show that IUD use does not increase the risk of abruptio placenta or placenta previa.

Significance to Biomedical Research and Program of the Institute: The findings from this study will improve the quantity and quality of information that is available to women and their physicians when assessing the benefit/risk characteristics of the IUD as a method of contraception.

Proposed Course: The project is completed, and the contract will terminate following publication of the findings; termination is scheduled for October 31, 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract:	A Prospective Study of the Effects of Induced Abortion on Subsequent Reproductive Function
Contract Number:	NO1-HD-6-2802
Contractor:	New York State Health Department
Money Allocated:	$10,112

Objectives: To evaluate the effect of induced abortion on subsequent pregnancy outcome by comparison of subsequent fetal death and live birth statistics for approximately 20,000 women with induced abortions recorded in upstate New York between July 1, 1970 and June 30, 1971 to fetal death and live birth statistics for an equal number of women with live births recorded during July 1, 1970-June 30, 1971 (the first year of legal abortion in New York).

Major Findings: When the outcomes of second pregnancies were compared for women whose first pregnancy ended in induced abortion vs. women whose first pregnancy ended in live birth, higher rates of short gestation/low birthweight infants, complications of labor and other problems were seen in the "induced abortion" group. However, when the outcomes of second pregnancies among the "induced abortion" group were compared to the outcomes of first pregnancies among women who had never undergone induced abortion, the rates of adverse outcomes were in general similar. Thus, termination of first pregnancy by induced abortion may delay the risks usually associated with the outcome of first pregnancy, but does not appear to increase those risks.

Significance to Biomedical Research and the Program of the Institute: This project has provided valuable information concerning the question of whether induced abortion damages subsequent reproductive capacity.

Proposed Course: The project has terminated.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title:	Prospective Study of the Outcome of Pregnancy Following Induced Abortion
Contract Number:	NO1-HD-6-2803
Contractor:	State University of New York
Money Allocated:	0

Objectives: To evaluate the effect of induced abortion on subsequent reproductive outcomes of pregnant women who have previously had one induced abortion (and no other pregnancies) to those of: 1) women who have previously had one spontaneous abortion; 2) women who have previously had one live birth; and 3) women who have never before been pregnant.

Major Findings: Women are being enrolled in the study from prenatal clinics at hospitals affiliated with the Downstate Medical Center of the State University of New York in Brooklyn, New York and from Queens General Hospital in Queens. Data have been collected for 550 pregnant women with one prior induced abortion, 250 with one prior spontaneous fetal loss, 900 with one prior live birth, and 1,000 with no prior pregnancies. These women are being followed to determine the outcomes of their pregnancies; additional women will be enrolled to the extent that time permits. When all pregnancy outcomes have been determined, the data will be analyzed to determine whether women with a history of one induced abortion (and no other pregnancies) have a higher than expected rate of adverse outcomes (spontaneous fetal loss, prematurity, and so forth) for their second pregnancies.

Significance to Biomedical Research and Program of the Institute: The effect of induced abortion on subsequent reproductive function and pregnancy outcome is an issue of considerable public health importance. This project is expected to provide detailed information concerning the effect of a single induced abortion on the outcome of second pregnancy. The project will thus complement the two more broadly focussed record linkage projects in this program area.

Proposed Course: The project is scheduled for completion in September.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Induced Abortion on Subsequent Reproductive Function and Pregnancy Outcome in Hawaii
Contract Number: NO1-HD-7-2801
Contractor: University of Hawaii at Manoa
Money Allocated: $69,973

Objectives: To evaluate the effect of induced abortion on subsequent reproductive function by linkage of state abortion records with live birth and fetal death statistics. Specifically, to study the effect of induced abortion upon rates of subsequent spontaneous abortion, premature birth, and other birth-related disorders.

Major Findings: Pregnancy outcomes which occurred between January 1971 and December 1978 are being compared for 16,500 women who collectively underwent 17,702 recorded induced abortions between July 1970 and June 1974 and two control groups: 1) all live births and fetal deaths which occurred in the State of Hawaii between January 1971 and December 1978, minus those which occurred to women in the "recorded induced abortion" group; 2) all live births and fetal deaths which occurred between January 1971 and December 1978 to women without a prior recorded induced abortion who are matched to the women with such a history for maternal age, racial background, residence (urban vs. rural), and birth order.

The preliminary analyses of these comparisons suggest a higher rate of spontaneous abortion and fetal death among women with prior recorded induced abortions than among women with no prior recorded induced abortions.

A follow-up study based on a sample of the total cohort study subjects is being conducted to evaluate whether loss to follow-up varies according to prior abortion history. Also, data on important potential confounding factors such as cigarette smoking will be gathered by direct interview of the subjects selected (by random sampling) for the follow-up study.

Significance to Biomedical Research and the Program of the Institute: This data linkage project provides a large data base from which to evaluate the effect of induced abortion on subsequent reproductive function. It complements the other data linkage project and the clinical cohort study in this program area.

Proposed Course: This contract has been extended to September 21, 1981 to allow completion of the follow-up study.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Birth Control and the Risk of Infertility
Contract Number: NO1-HD-0-2821
Contractor: University of Washington
Money Allocated: $480,700

Objectives: To evaluate the relationship between birth control practices and the occurrence of subsequent, undesired infertility by means of a population-based case-control study to be conducted in King County, Washington.

Major Findings: This project has just begun. The investigators plan to study all cases of non-congenital, female-factor infertility in married women 20-39 years of age that are identified from medical evaluations for apparent infertility in King County, Washington during a three year period. It is anticipated that approximately 1,500 such cases will be studied. For each case, one matched (for age, census tract, marital status, and pregnancy order) control will be studied. This will be a woman giving birth during the calendar year following the year that the associated case began trying to become pregnant. Cases and controls will be interviewed to obtain demographic, personal, medical, and reproductive history, and a detailed history of birth control practices. Data analysis will focus on estimating the relative and attributable risk of infertility for users of the intrauterine device, for women who have had induced abortions, and so forth.

Significance to Biomedical Research and Program of the Institute: Concern has been expressed that use of intrauterine devices, induced abortion, and possibly other birth control methods may increase the risk of subsequent, undesired infertility. This project will help to clarify this issue.

Proposed Course: The project is currently scheduled for three years.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: The Relationship Between Birth Control Practices and Subsequent Undesired Infertility
Contract Number: NO1-HD-0-2822
Contractor: Boston Hospital for Women
Money Allocated: $1,343,000

Objectives: To evaluate the relationship between birth control practices and the occurrence of subsequent, undesired infertility by means of a clinically-based, case-control study.

Major Findings: This project has just begun.

Proposed Course: Cases will be married women over 18 years of age who are identified as having female factor infertility at six clinical centers (New England Fertility and Gynecology Associates in Boston, U. of Colorado, George Washington U., U. of Kentucky, U. of Sherbrooke in Quebec, and U. of Vermont) over a two-year period. Controls will be of two categories: 1) women from infertile couples whose infertility is due to male factors, and 2) recently delivered women. Approximately 1,700 female-factor infertility cases, 1,700 male-factor infertility controls, and 5,600 recently delivered controls will be studied. Cases and controls will be interviewed to obtain data concerning demographic, personal, medical, and reproductive history, and a detailed history of contraceptive practices. Data analysis will focus on estimating the risk of female factor infertility for women who have used intrauterine devices, undergone induced abortion, etc. relative to the risk for women who have not used these methods of birth control.

Significance to Biomedical Research and Program of the Institute: Substantial concern has been expressed that use of the intrauterine device and possibly other methods of birth control may increase the risk of subsequent infertility. This project will provide a quantitative assessment of this issue.

Proposed Course: It is anticipated that there will be a six month development phase, during which the study protocol and questionnaire will be refined. This will be followed by two years of data collection. Finally, six months will be devoted to data analysis and the preparation of reports.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Center for Population Research

The Center's grant and contract program in the behavioral-social sciences focuses on the size, rate of growth, and composition of our nation's most important asset, its population. The topics addressed by specific research projects derive from fundamental questions concerning the growth and distribution of our population, and their relation to individual and societal welfare. For example: What accounts for changes in the number of children couples decide to have? How successful are they in controlling their reproduction? What are the consequences of many or few children for individuals, families and the nation? What are the effects of age at birth and birth spacing on mothers and their children? How does internal migration affect the welfare of the people moving and the communities from which they come and to which they go? How would a rise or decline in the rate of population growth rates from a combination of natural increase and net immigration affect our economy and our environment in the future? Answers to these and many other questions are needed in order to deal with population changes now and in the years ahead. The results of research are intended ultimately to help individuals and couples to understand the personal and societal consequences of their behavior and to help the government evaluate the impact of a variety of policies and programs on both the quantity and quality of our human resources.

The staff of the Social and Behavioral Sciences Branch uses grants to support research on these broad objectives, and uses contracts to support research on more specific research goals identified by the Institute. The Branch's strategy has been to concentrate on topics of current societal importance as well as questions of scientific concern. Consequently, the program has emphasized not only such topics as social and economic factors affecting the level and trend in national birth rates, but also methods of fertility control used, effectiveness of contraceptive practice, changes in family size over time, the consequences of family size, the determinants and consequences of adolescent fertility, and the implications of immigration for the U.S. One result of this strategy is that the Branch's research program changes in response to changing social conditions as well as in response to developing scientific knowledge. At present, its program focuses on the topics listed below:

Trends and Variations in Fertility

There has been considerable progress in the search for an economic model to explain the rapid changes in fertility that have occurred over the last three generations. An economic model of fertility relates childbearing to changes in the resources available to families (income) and to the cost of childbearing (price). As income rises, childbearing should increase, and as the price of childbearing increases, childbearing should decrease. A number of studies have proposed ways to measure these relationships. One study has emphasized the importance of including the opportunity costs of childbearing (as measured by wife's wages) in calculating the overall price of childbearing. In this study, Dr. Butz reports that when opportunity costs are included in the economic model

of fertility, the model gains considerable strength in explaining recent swings in fertility. In another study, Professor Richard Easterlin has hypothesized that income should be measured by the amount of change in perceived economic opportunities for persons in their prime reproductive years relative to the aspiration levels of these persons established in their youth. When these opportunities are relatively high, relative income is high and encourages earlier marriages and increased childbearing. A recent study by Dr. Devaney has suggested that models incorporating relative income measures and female wage rates as proxies for opportunity costs can combine strongly to influence fertility and female labor force participation. Higher relative income appears to increase completed fertility and to decrease female labor force participation. Also, higher relative income appears to induce both earlier childbearing and earlier female labor force participation. This study suggests, however, that female wage rates were the most important factor in recent fertility trends and trends in female labor supply. Increased female wage rates, mainly during the past 20 years, are associated with lower completed fertility, longer postponement of childbearing, and increased labor force participation. This study does suggest, however, that Easterlin-type models focusing on income effects and Butz-type models emphasizing price effects can be reconciled comfortably within a classic economic model of fertility.

Recent work by Easterlin and Wachter suggests that an economic analysis of fertility must also develop a comprehensive picture of the socioeconomic effects of fertility swings. They argue that because of diminished immigration, affecting the supply of labor, and recent monetary and fiscal policies which have maintained a high and growing level of labor demand in post-war America, the effects of swings in fertility on relative cohort size could for the first time play a central role in affecting a cohort's life chances. In other words, they maintain that for the first time in American experience, cohort size has come to play an important role in shaping the socioeconomic experience of different cohorts. Easterlin and Wachter advance a "supply side" analysis of rising unemployment combined with accelerating inflation during the period since 1960. They argue that the growing abundance of younger workers that has occurred since 1960 tends to increase the economy-wide average unemployment rate, in general, and the age specific unemployment rate, especially for younger workers, in particular. They argue that modern monetary and fiscal policy has not correctly taken into account the effect of uneven cohorts of workers on the overall supply of labor, and has created a demand for labor that does not correspond with the altered mix of the labor supply. Hence the extra demand creates relatively little improvement in employment conditions, especially for younger workers and spills over into price increases. This contributes to the phenomenon of rising unemployment coupled with accelerated inflation known as "stagflation." They also argue that changing cohort size can account for important changes in marriage, homicide, suicide, illegitimacy, and divorce. Lastly, they argue that fertility swings may be related to social and economic conditions through the mechanism of changing cohort size and may combine to create a 40-year "new Kuznets cycle," in which small cohorts produce large cohorts and large cohorts produce small cohorts with attendant swings in social and economic conditions.

For the past twelve years, CPR has supported an analysis of fertility variation in Taiwan. In this study, Ronald Freedman has documented the remarkable changes

in fertility occurring in Taiwan as the process of modernization proceeds at a rapid rate. An important factor accounting for the dramatic fertility decline in Taiwan is the increase in the age at marriage which, in turn, is largely a function of changes in the educational distribution rather than of changes in nuptiality within educational groups. This study also found that breastfeeding had contributed to reduce the probability of conception even after the resumption of menstruation. In addition, this study has shown that changing preferences about the number of children has important predictive validity in regard to subsequent fertility. A strong desire to limit fertility has contributed to a strong desire for effective contraception. The study indicates that there were high levels of contraception, contributing to low fertility of contraceptive acceptors over a long period of time. For example, an analysis of IUD users in Taichung indicates that, over the period 1973-1975, 79 percent of the original IUD acceptors were using some form of contraception although only 17 percent were still using the IUD. Recent work has endeavored to examine the micro-level routes of modern reproductive behaviors in Taiwan seeking especially to uncover the intervening links between increased education and low fertility. Dr. Freedman argues that preferences for smaller families are related to a complex of attitudes and behaviors generally associated with modernization: a larger role for wives in family decision making, more activity outside the familial contacts, less expected dependency on children, and less observation of traditional kinship-based religious ritual.

Factors Affecting the Control of Fertility

In the continuing search for psychosocial factors affecting fertility regulation, an investigator is testing the hypothesis that the family building process is a developmental one. It is postulated that there is a sequence of events in which fertility values, the criteria used in evaluating family size, are both determinants of and determined by the number of children added to the family. The cross-sectional phase of the study has shown that in the initiation of family building, perceived costs, rather than rewards, of children are most closely related to family size. The arrival of the first child is found to be a critical event, changing perceptions more than does any additional child. Further, with the experience of each additional child, there are changes in the relationships between fertility values and desired family size. Thus, the developmental hypothesis concerning changes of parental evaluations of family size is supported, but the completion of the longitudinal aspects of the study will throw much more light on motivational factors involved in the building of families.

An analysis of spouse interaction yielded findings which suggest that many couples, especially those with children, experience conflict and uncertainty about the decision to have or not to have a child in the next two years. Wives generally expected a future child to demand much more of their time and energy than did their husbands, but childless couples perceived their future child-care roles as more nearly equal than did parents. It seems that some couples had an unrealistic expectation of child-care roles before the birth of their first child. The decision to have or not to have a child in the next two years depended on both spouses' perceived rewards and costs of parenthood, as well as the wife's age and her perception about the financial necessity of her employment. However, wives' perceptions were clearly more important than husbands' in predicting fertility.

Analysis of data from a longitudinal study points to seven aspects of couple interaction that are relevant to reproduction: communication, conflict, influence, decision making, bonding, division of labor, and adaptation to change. The first four directly affect the psychological sequences that lead to having a child. Marriage and marital dissolution are affected by bonding. Division of labor is reflected in female role orientation and behavior.

Still other studies attest to the complicated interplay of the determinants of fertility. We are fortunate to have a study of changing expectations and preferences for family size over a fifteen-year period (1962-1977). This investigation showed considerable lowering in these preferences and expectations during this period which reflects the gradual change of U.S. attitudes and behavior regarding family size. Still, prior family size expectations and preferences as well as the discrepancy between husband's expectations and wife's preferences help explain the relationship between completed family size and initial expectations. Shifting preferences play a major part in reducing expectations, while having unwanted children is strongly associated with having a completed family size that exceeds initial expectations.

Whether or not a woman is gainfully employed is yet another facet of the complex mosaic of factors affecting fertility. A National Fertility Survey recently found that female labor force participation can depress fertility through the mechanism of competition for a woman's personal resources. Women who work between marriage and the birth of their first child are likely to delay that birth. Working after the first birth also delays the second. Opposite forces also operate: many women become trapped between accidental childbearing and financial pressure. The cumulative effect of growing family size is to depress labor force participation, but after two or three children, the financial burden creates significant counterpressure favoring employment. A somewhat related finding from a longitudinal study (1962-1977) indicates that additional children and favorable earnings of the husband were associated with less employment of the wife, while increased education and divorce were associated with greater female employment. That these phenomena are not new in the U.S. is demonstrated by data obtained from women born in 1901-1910. In the case of these cohorts of women, the delay in childbearing within the first birth interval has a strong positive association between education, husband's white collar occupation, and the wife's working within the interval.

The psychosocial factor of alienation, defined as feelings of normlessness, meaninglessness, and social isolation, has been found to interfere with rational family planning and fertility control. Comparatively speaking, subjects high in levels of alienation tended to terminate their education at lower levels, to marry at earlier ages, to be less knowledgeable about sex and reproduction, and to use less effective contraceptive methods, but to see fewer advantages of having children. The National Fertility Survey showed that religion is a major variable affecting planning concerning fertility and fertility regulation. Catholics had 26 percent higher fertility than non-Catholics, made up of 22 percent higher number of intended pregnancies and 39 percent higher number of unintended pregnancies.

Several studies have contributed to our understanding of contraceptive practices, one of the factors directly related to fertility behavior. Findings from a

longitudinal study indicate that the success of contraception depends both upon the type of contraceptive used and on how regularly it is used. Women tend to change their contraceptive methods frequently, in part because each type of method has specific disadvantages associated with it. A number of psychological factors including motivational, cognitive, and attitudinal mechanisms, as well as personal style and stress, are involved in ineffective use. These psychological factors tend to be different for married and unmarried women.

The National Fertility Survey analysis of women using the pill or IUD during the period 1970-1975 revealed an increase of discontinuation related to problems encountered during use. The findings indicated that there is a distinct association between the amount of media coverage of these methods and the dropout rate, with a cumulative effect evident in the case of the pill. The study also pointed out that 68 percent of non-Catholics used contraception to delay an intended pregnancy, while 58 percent of Catholics used contraception for this purpose. Differences between these groups were also found for failure rates. For example, the fertility rate when contraception was used to delay pregnancy was 67 percent higher for Catholics than for non-Catholics. Another investigator discovered that the effectiveness of the contraceptive used at first intercourse of married couples was associated with educational attainment, age at marriage, meaninglessness, and traditionalism. Current contraceptive risk-taking across various levels of parity was positively related to meaninglessness and traditionalism, and negatively related to wife's plan for employment, and effectiveness of contraceptive at first intercourse after marriage.

The control of fertility is not such a new practice, as noted in a study of women born in the early 1900's. This cohort (1901-1910) bore their children during the Depression and had very low completed fertility. Seventy-one percent of women born during 1901-1910 reported that they or their husbands had used a method defined as modern, i.e., condom, diaphragm, jelly or cream, suppository or tablets, vaginal cap or IUD, contraceptive douche, or sterilization, and 49 percent had initiated use before the first birth. The median length of the interval to the first birth was more than twice as long for contraceptive users (33 months) as for nonusers (14 months).

In a study in Scotland, it was found that, from 1953 to 1977, substantial increase in childbearing among unmarried women under age 20 was accompanied by a nearly equal increase in induced abortions. The number of pregnancies among married women decreased markedly, and the number of births decreased even more so, due primarily to increased sterilizations. The proportion of pregnancies resulting in live births declined over the twenty-five-year period; by 1973-77, it was less than seventy-five percent.

The National Fertility Survey indicates that sterilization is becoming the major method of limiting family size in the U.S. It is estimated that between two-thirds and three-quarters of once-married white couples will obtain sterilizing operations within fifteen years after their last wanted birth. In a study of couples in the early years of marriage, 78 percent reported plans for sterilization after achieving their desired family size. Couples who agreed on having no plans for sterilization tended to have low levels of knowledge about sex and reproduction, high levels of traditionalism, and expected family sizes of three or more children. The data also showed a close correspondence between

plans for sterilization and attitudes toward abortion. Couples who agreed on plans for sterilization were likely to be tolerant of abortion, to be knowledgeable about sex and reproduction, and to expect relatively few children.

Puerto Rico is one of the developing areas with the longest history in the development of fertility regulation services, particularly sterilization. A current study shows that female sterilization has increased between 1965 and 1976 from one-third to thirty-seven percent of married women. In 1976, an additional eleven percent were taking oral contraceptives. The pill has not yet displaced sterilization in Puerto Rico, but has displaced less effective methods and increased in use.

Consequences of Adolescent Pregnancy and Childbearing

Concern about early pregnancy and childbearing revolves around the effects on the young woman, her child, the father, and other family members involved, as well as society as a whole. While birth rates of older adolescents have declined in recent years, birth rates have increased among younger adolescents. There has been an increase in the likelihood of out-of-wedlock births, and adolescents still account for one-third of the legal abortions performed in this country each year. There is substantial evidence of high levels of unintended pregnancy and childbearing among adolescents, and research is generating an improved understanding of the effects of this behavior.

In previous years, the effects of early childbearing on children's early and school-age development have been reported. Early childbearing is also associated with reduced educational attainment, increased fertility and faster pace of childbearing, marital instability and negative economic prospects for women. Among high school graduates, the effects of early marriage and parenthood on educational expectations were small relative to many other influential factors (ability, family background, etc.). Among married adolescents who had finished high school, parents differed little from nonparents in educational expectations when background characteristics and marriage dates were controlled. In short, young people with rather low educational expectations appeared to be self-selected into adolescent parenthood. A study based on the National Longitudinal Study of high school seniors found young women's orientation toward work was closely related to their marital and parenthood status. The importance of careers to married women decreased near the marriage date and decreased further when parenthood was imminent. Married men, compared with unmarried men, attached somewhat more importance to their careers, but parenthood had only a slight effect on men's orientation toward work. Among married women, mothers seemed slightly less satisfied with their career progress than did non-mothers, and dissatisfaction seemed linked to the timing of parenthood. Although the pattern was similar among married men, the timing of parenthood was not important.

Early childbearing also has impact on society, for when individuals do not achieve their educational and occupational goals, society loses their contributions to the economy and the tax base. More directly, if early childbearing leads to greater use of public services, there is a direct impact on public expenditures. These public sector costs include Aid to Families with Dependent Children (AFDC), Medicaid, food stamps, foster care, and so forth. Several research projects are addressing this question and are assessing the effects of early childbearing

as distinct from other personal characteristics of the women.

Determinants of Adolescent Fertility Behavior

Research on the determinants of adolescent fertility and related behaviors covers a wide range of methodological approaches and issues. One study is looking at very early developmental events and their relationship to fertility-related behaviors. Subjects range from preadolescents with no boyfriend or girlfriend relationships to those with some early heterosexual experience. There appears to be little relationship between early heterosexual behaviors and use of beer, wine, cigarettes or marijuana. There was also no relationship with pubertal development, but there were differences by income level and general achievement measures (WRAT scores). The preadolescents with no experience and no boyfriend or girlfriend were predominately middle income with higher achievement scores.

A four-community study focused on 16 and 17 year-old males and females and the relationship between psychosocial factors and fertility-related behaviors. Psychosocial factors predicted sexual behavior better than contraceptive behavior or pregnancy but were difficult to classify as antecedents or consequences of sexual behavior. Not surprisingly, the best predictors were those that closely corresponded to behaviors. For example, approval of sexual activity for adolescents in love most strongly predicted sexual behavior, but again the attitude appeared to follow the behavior rather than precede it. Willingness to have unprotected intercourse and perceived barriers to the use of contraceptives predicted contraceptive non-use. In a separate study other psycho-social measures appeared related to contraceptive side effects and failures. Adolescents initially assessed as: (1) reporting more negative family relationships earlier in life; (2) showing lower levels of socialization and generally displaying more socially unacceptable behavior; (3) having less ability to think ahead into the future; (4) having a poorer self-concept; and (5) displaying other negative personal characteristics experienced more side effects and more side effects that led to contraceptive failure. Also, medical problems generally were related to personal characteristics. Demographic characteristics such as age, ethnic background, and class were studied in relation to contraception, but were found as a rule not to be related systematically to contraceptive side effects. These findings suggest a role for psychological determinants of what are usually considered physiological problems in the use of contraception. The study results have important implications for the prediction and prevention of contraceptive failure, and have additional relevance for general health care and public policy.

A theme in many of these studies is the disentanglement of personal traits that relate to sexual and contraceptive behavior from social-structural factors. The Kantner/Zelnik studies have addressed the factors associated with non-use of contraceptives and found increased but not extensive use of effective contraception by unmarried adolescent females. The major reason given for non-use of contraception was the belief that pregnancy could not occur, and another important reason was that intercourse was unexpected. About 20 percent of the women in the national probability study had had intercourse by age 16 and 66% by age 18. Premarital intercourse was more prevalent among blacks, older adolescents, women of low socioeconomic status, non-Catholics, and women not reared by their natural parents. Whites had more premarital sexual partners than blacks, and the

longer a woman was sexually active the more partners she had. Plans to marry, use of medical contraceptive methods, and number of premarital sexual partners were directly associated with high frequency of intercourse; frequency was higher for whites than for blacks. Age at menarche was directly related to age at first intercourse and inversely related to the prevalence of premarital intercourse. Most teens were at least two years post-menarche when they became sexually active, even the girls who were sexually active quite young. These data were used to assess the risk of conception soon after the initiation of sexual activity and found it quite high. While most teens delay coming for contraceptive advice or services, their risk of becoming pregnant is high in the first six months of exposure to intercourse and half of the premarital pregnancies occur during this time. The younger the adolescent the greater the risk because contraceptive practice is worse for the younger teens. One implication of this analysis is that efforts should be directed at reaching adolescents before they become sexually active and before they identify themselves as being in need of services.

Preliminary analyses of interviews with a sample of teenagers from an urban area has shown significant differences in educational aspirations, plans for motherhood, and problem solving skills among four categories of young women: pregnant adolescents, sexually active non-contraceptors, sexually active contraceptors and virgins. Pregnant adolescents are much less likely than others to want to attend college and are more likely to have fallen behind in school, even before becoming pregnant. Non-contraceptors want their first child at a younger age than contraceptors or virgins; fifteen percent of the non-contraceptors want their first child at age 17 or 18. Contraceptors show more ability than non-contraceptors or pregnant adolescents to plan for personal goals and to find alternative solutions for personal problems. The influence of the parents can be seen in the association of mothers' low aspirations for their daughters' educations and the daughters' failure to practice contraception. Daughters of employed mothers are more likely than daughters of housewives to know about birth control and the negative consequences of pregnancy, to have higher educational aspirations, and to use contraceptives. A study based on the 1971 Kantner-Zelnik data shows that educational expectations were negatively associated with the probability of intercourse; however, in the subsample of sexually active women, educational attainment and expectations were not related to contraceptive practice or to the incidence of pregnancy. Family background was strongly associated with the probability of pregnancy and also was related to sexual activity and contraceptive practice. Parents' educational attainment and family income were negatively related to the probability of pregnancy.

A study of adolescent women in Ontario, Canada found that age, religiosity, career aspirations, parents' acceptance of premarital intercourse, peers' acceptance of premarital intercourse, peers' experience with premarital intercourse, commitment to dating, and frequency of dating were related to whether a young woman was a virgin. Peers' experience with premarital intercourse was the most important predictor of virginity status, followed by commitment to dating and religiosity. The influence of parents can be seen in the analysis of factors associated with embarrassment in obtaining contraceptives. Embarrassment was greater near the parental home than distant from it. Least embarrasment was found for obtaining birth control pills and most for condoms among this female sample. Guilt about sexual activity and ease of access to contraceptives were among the most important influences on embarrassment or the absence of it. Parents' attitudes toward

premarital intercourse had the most important effect on embarrassment about obtaining contraceptives near the parental home, but away from home, friends' attitudes were more important.

One study has followed young women who gave birth as adolescents and looked at the correlates of second pregnancies among adolescents. Preliminary findings indicate that adolescents who became pregnant again, compared with those who did not, were more likely to be married, younger, welfare recipients, less intellectually competent, to have had a prior pregnancy terminated by abortion, to have worked less, and to have had more problems using contraceptives. Of the first 40 repeat pregnancies studied, all but seven were terminated by elective abortion; most of the seven babies carried to term were unplanned and undesired.

Other research has addressed the impact of hospital and clinic policies regarding health care as well as State and federal policies regarding welfare on adolescent fertility behavior. One analysis used the 1976 Survey of Income and Education data to test a presumed direct effect of family planning services, abortion availability, age of consent laws concerning contraception and abortion, Medicaid coverage of abortion, welfare benefit levels and acceptance rates, welfare coverage of unborn children and unemployed father, and benefit levels of several programs on adolescent childbearing. Indirect effects were tested for local unemployment rates, female wages and educational opportunity. State and federal assistance programs did not have a positive effect on adolescent childbearing. In fact, measures of benefits from Aid to Families with Dependent Children (AFDC), when statistically significant, were consistently negative, and AFDC policy variables were consistently unimportant. However, analysis of abortion data indicated that greater availability was associated with lower fertility.

Preliminary multiple regression analysis for the U.S. showed that on a cross-sectional basis (1970 and 1975), areas with higher proportions of adolescents enrolled in family planning programs were also areas with higher adolescent birth rates. This is a common finding in many countries, due to the location of clinics in high fertility areas and to differences in socioeconomic characteristics and levels of sexual activity. As expected, early findings showed that areas with greater increases in the proportion of adolescents enrolled in family planning clinics between 1970 and 1975 generally had greater decreases in adolescent birth rates.

The analysis of the Survey of Income and Education data found a fairly consistent relation between higher female educational achievement and lower adolescent fertility. In this analysis, the availability of sex education did not affect fertility, but a more refined measure of availability is needed. In an individual-level analysis, sexual and contraceptive knowledge showed the strongest association with fertility behavior. Women who knew when they were likely to conceive and knew about medical methods of contraception were more likely to be sexually active and to use contraceptives. However, it was not possible to ascertain whether knowledge preceded sexual activity and contraceptive practice or vice versa. In a study of health facilities, adolescents reported that in choosing a physician, they are constrained by cost; fear that information will not be kept confidential; and anticipated lack of privacy during the physical examination. Concerns voiced by teens about communicating with physicians are that they do not consider adolescents as people with feelings and rights; give inadequate

explanations; are judgmental; and ask excessively personal questions. Adolescent females characterize as ideal a physician who encourages questions, gives good explanations, and helps the adolescent to relax; is neither too old nor too young; is female; is friendly and sincere. Problems with parents that affect adolescents' reproductive health care include the need to have information about their sexual activity kept confidential from parents; need for parents to make their medical appointments; and need for financial support from parents. Adolescents also identified the need for more and better sex education. Another study which sought to elicit teens' views regarding problem pregnancies concluded with three major recommendations: (1) greater parent initiatives, perhaps in discussing problems of teenage sexuality and pregnancy with teenagers not their own children; (2) improved development and marketing of an effective nonprescription contraceptive meeting teenagers' needs, and (3) improving school sex education programs by placing more emphasis on women's needs, assertiveness training, and communication problems with males, particularly about contraception.

Consequences of Family Size

As several studies cited above have indicated, the anticipated consequences of having a child or an additional child is an important factor determining fertility. Research emphasis in recent years has been on the consequences of childbearing and childspacing for both parents and children so that couples planning their childbearing patterns as well as those who counsel them and provide health services for them may have more information at hand. Several studies conducted in the recent past have investigated the consequences of childbearing and childspacing on child development and achievement. These studies were completed and reported. Now a major program initiative is under way on the consequences of low fertility (the effects of childlessness and of one-child families) on the adults and children involved. There are three reasons for this initiative. The first is that there has been practically no research to date on the consequences of low fertility; the second is that low fertility is increasing; and the third is that there is reason to believe that many people have a child or have more than one child at least partly in the belief that there are negative effects on the couple and/or the child for not doing so. Several studies are under way to address these issues, using research covering a wide range of factors from psychological functioning to economic attainment, and taking into account such important variables as age (of the couple or of the child), sex of the child, the degree of intendedness of the family size, and so forth.

Four studies of only children using large, extant data sets show no adverse effects of being an only child. Only children were found to be more like first-borns in larger families, and were shown to be slightly superior on cognitive abilities and achievement to children with siblings. On the whole, "onlies" simply did not differ from children with siblings on a wide range of factors including physical development, utilization of medical care, behavioral and psychological factors, marital status, number of children they had, divorce rates, occupational choice, or levels of income. These studies show no negative effects of being an only child. A study which compared "onlies" with children with siblings in one-parent households also showed no negative results of being on "only." In fact, "onlies" from these one-parent households showed fewer interpersonal, emotional, and behavioral problems and were more mature, independent, and comfortable with adults than comparable children with siblings.

One study comparing "onlies" and firstborns at 3, 12, 24, and 36 months of age showed some differences at 3 months in social behavior (with "onlies" crying more and smiling less), but these differences disappeared by 12 months and did not reappear. The reasons for the difference found at 3 months were not clear, but it was noted that the percentage of abnormal pregnancies and deliveries was slightly higher for mothers of "onlies" in this study. This same study found that the birth of siblings adversely affected the cognitive performance of firstborns aged 24 months or less. Since the consequences of family size may vary with age of the child, two new projects are doing in-depth studies of adolescent only children and comparing them to first and lastborns in larger families. These studies are concerned with the consequences of being an "only" on intelligence, interpersonal orientation, attitudes, time use, reciprocal interaction skills, and ego identity formation.

Likewise, family size may have different effects for parents and children. Several studies are under way which compare the consequences of having, 0, 1, and 2 or more children on a variety of factors such as social adjustment, psychological well-being, perceived quality of life, physical health, occupation, education, and marital stability. Three of these studies have preliminary findings which show that, for wives, having children is generally negatively related to continuity of employment as well as to occupational aspirations and attainment. Childless wives are more likely to have uninterrupted employment than all other wives, and mothers of only children are more likely to have continuity of employment than wives with more children. In one of these studies, occupational aspirations were measured at age 16 and again at age 40, and while all the women had similar occupational aspirations at 16, childless women had significantly higher occupational mobility aspirations at age 40 than did either the mothers of only children or mothers of two or more children. Another of these studies shows that living standards differ most as family size increases above 3 children. Consumption patterns of childless and small families differ somewhat from larger families, but the primary difference as family size changes appears to be in use of time.

Four new studies are under way on the economic consequences of family size. One is concerned with the difference family size makes on level of living, and the other three are attempting to estimate actual expenditures on children in families of various size, income, age, residence, and occupation. These studies will also predict, in constant dollars, the cost of raising a child born in 1980.

Several new studies are concentrating on the consequences of both involuntary and voluntary childlessness. These studies are looking at the effects on men, women, and married couples at different points in the life cycle (including old age) and are assessing the relationship of childlessness to educational and occupational attainment, social and economic well-being, measures of personal and marital satisfaction, and indicators of quality of life.

Migration and Population Distribution

Migration patterns often have as much, or more, influence on the size of population at the local level than does fertility. Studies have looked at both interstate and intraurban movements, assessing their determinants and consequences, as well as research on international movements of people. Research now under way

is focused on the relationship between population movement and economic issues and also on the individual decision making involved in such moves. One study is examining the relationship between the nature of internal migration and the level of socio-economic development in several countries. It builds on an analysis of 1970 U.S. data which indicated that most interstate migrants are moving for at least the second time, and that those who are going to a third state of residence (i.e., not going back to their state of origin) are a highly selected group, differing from both those who are leaving the home state and those who are returning to it.

In regard to international migration, a longitudinal study considered factors affecting the decision of the 1970 cohort of Filipino graduate students in the U.S. to either return home or become U.S. immigrants, and the consequences of returning home versus immigration. A sociopsychological model of the determinants of international skilled migration was based on seven studies conducted on the brain drain from the Philippines to the U.S., and encompassed demographic, sociopsychological, environmental, and mobility factors. One-sixth of the annual number of professional, technical, and related workers coming into the U.S. over the past decade have been Filipinos, a majority of whom have been medical personnel. A large proportion of the others originally entered the U.S. as students. Returnees were slightly older than immigrants, but there were no differences in terms of sex or socioeconomic status or in academic aptitude and educational accomplishments. Although a higher proportion of returnees were single, those who were married had more children than did immigrants (2.45 versus 1.69), reflecting the greater ease of raising children in the Philippines. Returnees on the whole were more satisfied with their jobs, freedom from discrimination, and presence of close friends and relatives. Immigrants reported greater satisfaction with the political and economic climate, their sense of independence, the family's economic well-being, and educational opportunities for their children.

Presently, legal immigration accounts for one-fourth of the yearly population increase observed in the U.S., and illegal immigration is estimated to contribute even more. Current estimates of legal and illegal immigration, coupled with the present low fertility rates, would result in a U.S. population in the year 2000 very similar to that obtained from a higher level of fertility without illegal immigration. A number of projects submitted under the Request for Applications on "Implications of International Migration for the United States" have been funded. Two studies concern the fertility of immigrants generally and of Mexican-Americans in particular. Three deal with the demographic and economic consequences of immigration, earnings and labor force behavior of immigrants, and labor market effects of undocumented workers. Six address the question of adaptation in one form or another: the adjustment and impact of recent immigrants, Asian-Americans, socioeconomic status of mothers of Mexican origin, the work and community absorption of Mexican illegal immigrants, Dominican migration, and Finnish family adaptation. In addition, one project plans to develop a public use file regarding the resettlement experiences of Indochinese refugees.

Workshops

A contractors' workshop on the Economic Determinants of Fertility was held on 11 March 1980. Six investigators working in this area of research described their progress, problems and findings. The workshop served to coordinate

methodological efforts of the investigators at that stage in their progress in order to achieve the greatest comparability of results and inform investigators of work being done by others in related areas.

A workshop on the Effects of Adolescent Childbearing on the Child was held on 24 September 1979 to bring together researchers who were studying the effects of adolescent childbearing on the child. Six investigators reported their progress and discussed their findings. The discussion of the general area of research on adolescents and their children focused on the role of the family and variations in family structure in mediating effects for the child. The discussion was particularly useful for planning future research initiatives.

A contractor/grantee workshop on the Determinants and Consequences of Adolescent Fertility Behavior was held 3-5 March 1980. Twenty-eight investigators working in related areas described their work and findings. The workshop provided staff with an up-to-date overview of all research funded under the grant and contract mechanisms on the subject of adolescent fertility behavior and informed investigators of work being done by others in related areas.

Targeted Research

Requests for Contract Proposals (RFPs)

An RFP for Research on the Societal Consequences of Adolescent Childbearing (RFP-NICHD-SBS-80-9) requested proposals for research on the public sector costs associated with adolescent childbearing. The research is intended to supplement studies of the consequences of adolescent childbearing to the mother, child, father and family members, and studies resulting from a previous RFP for research on public sector costs of early childbearing.

Research contract proposals were also solicited for research on the Demographic Analysis of Family and Household Structure (RFP-NICHD-SBS-80-12). Changes in fertility, mortality, migration, nuptiality and the patterns of family and household structure exert significant influences on society and the economy. Recent evidence in the U.S. and other developed nations indicates that new patterns of family and household structure are emerging. The proposals requested in this RFP are designed to study these changes.

An RFP for Research on the Components of Effective Fertility Regulation (RFP-NICHD-SBS-80-13) requested proposals to address the effectiveness of methods of fertility regulation as they are commonly used. The proposals differ from the studies which determine theoretical effectiveness through controlled clinical trials, by developing studies of effectiveness in real life situations. Social, psychological, economic, demographic, health, and other factors may influence the selection, use, and effectiveness of fertility regulating methods. The research will analyze interrelationships among characteristics of users, fertility regulating methods, and delivery systems in the effective control of fertility.

Institutional Programs in Multidisciplinary Population Research

The Social and Behavioral Sciences Branch support three kinds of Institutional

Programs in Population Research: Program Projects, Population Research Centers, and Specialized Population Research Centers at major institutions in the United States. Support for these institutional programs enables coordinated, highly skilled groups of behavioral-social scientists in a variety of disciplines to organize and conduct multidisciplinary population research programs. These programs are designed to attack complex population problems that cannot be adequately studied by an individual investigator working alone.

A. Population Research Centers (A Population Research Center grant provides for centralized services and facilities required for enhancement of the quality and productivity of extant population research projects.)

Population Research Centers supported by SBSB, together with their major research areas, are located as follows: (1) University of Wisconsin at Madison-demography and human ecology; (2) University of Texas at Austin-demography of minority groups and population change and distribution; (3) The Johns Hopkins University-demography, teenage fertility, and reproductive biology; (4) Princeton University-demography, population economics and statistics; (5) University of Washington-fertility, mathematical demography, and human ecology; (6) University of Michigan-fertility, population distribution and differentiation, and economic demography; (7) University of Pennsylvania-fertility, population movement, and economic demography; (8) University of North Carolina-fertility, family planning, migration, and population statistics.

B. Specialized Population Research Centers (Specialized Population Research Center grants provide for the support of a comprehensive population research program that is specifically responsive to research areas specified by the CPR.)

The SBSB is funding two Specialized Population Research Centers. The Center at the Rand Corporation has two major foci: (1) the demography of families and households and (2) regional and local population changes. The Center at the National Opinion Research Center specializes in the economic demography of the family.

Population Research Manpower Development

A. Institutional Fellowships

The NICHD awards grants for the support of institutional behavioral-social population research training programs, and for the support of behavioral-social predoctoral and postdoctoral trainees. During fiscal year 1980, the NICHD awarded population research training grants to 12 behavioral-social programs at the following institutions: Princeton University; University of Wisconsin at Madison; University of Michigan, Economic Demography; University of Michigan, Population and Human Ecology; Brown University; University of Texas at Austin; Florida State University; University of Pennsylvania; Yale University; University of Chicago; University of North Carolina, Population Studies; University of North Carolina, Population Statistics.

B. Individual Postdoctoral Fellowships

Four behavioral-social postdoctoral fellowships were awarded during fiscal 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	A Study of Couples' Motivations for Parenthood, Decision-Making, and Fertility Regulation
Contract Number	:	NO1-HD-52807
Contractor	:	University of California, Los Angeles
Money Allocated	:	$125,209 (1975); $62,925 (1977); $142,463 (1978); $133,915 (1979)

Objectives: This project examined the relationships among motivations for parenthood, division of power and responsibility in the marital dyad, and fertility behavior. Six hundred couples, with wives aged 15-34, were interviewed twice during a three-year period. Half the couples were recently married for the first time, and the other half recently had a first child. In each subgroup, one member of each couple--half the husbands and half the wives--was designated as a primary respondent from whom most of the information was collected.

Major Findings: Preliminary findings showed that spouses who disagreed about the number and spacing of children wanted larger families, less frequently discussed family size and sexuality, and less frequently communicated their feelings than did other spouses. For recently married couples, disagreement about fertility was associated with full-time employment, high education, and high socioeconomic status of the wife. Among couples with a child who had disagreed about fertility at the time of marriage, wives were more likely than husbands to have revised the total number of children wanted. The reverse was true for newly married childless couples.

Subsequent analysis suggested that many couples, especially parents, experienced conflict and uncertainty about the decision to have or not to have a child in the next two years. Wives generally expected a future child to demand much more of their time and energy than did their husbands, but childless couples perceived their future child-care roles as more nearly equal than did parents. This finding suggested that some couples had an unrealistic expectation for child-care roles before the birth of the first child. The decision to have or not to have a child in the next two years depended on both spouses' perceived rewards and costs of parenthood, as well as the wife's age and her perception about the financial necessity of her employment. However, wives' perceptions were clearly more important than husbands' in predicting fertility.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interest in the antecedents, determinants, and correlates of fertility.

Proposed Course: It is anticipated that the study will be completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Longitudinal Study of Intended and Actual Fertility: The 1975 National Fertility Study
Contract Number: NO1-HD-52819
Contractor : Princeton University
Money Allocated: $589,950 (1975); $228,945 (1976 TQ); $230,614 (1977); $215,990 (1979); $214,398 (1980)

Objectives: Toward the end of 1975, some 3000 white women who were interviewed in the 1970 National Fertility Study, specifically all those married at less than 25 years of age, and not before 1951, whose first marriages are still intact, were reinterviewed, along with some 1000 white women of comparable marital status and age at marriage who married during the years 1971-75, to collect complete pregnancy histories and a record of use of the various modes of fertility regulation (contraception, abortion, and sterilization) vis-a-vis each pregnancy. Data collected consisted of both retrospective and prospective fertility intentions, attitudes toward reproduction and modes of fertility control, as well as information of explanatory value concerning such topics as experience in the labor force, attitudes toward sex roles and population problems, etc.

Major Findings: This study clearly indicates that sterilization is becoming the major method of limiting family size in the United States. It is estimated that between two-thirds and three-quarters of once-married white couples will obtain sterilizing operations within 15 years after their last wanted birth. The study also shows that the number of births intended, desired, and regarded as ideal all declined substantially between 1970 and 1975. In addition, improved control of fertility has reduced the incidence of unintended births. These factors help to explain the current low level of fertility in the United States. The findings support the conclusion that working can have the effect of depressing fertility through the mechanism of competition for a woman's personal resources. Women who worked between marriage and the birth of their first child were likely to delay that birth. A similar delay was also evident for the second birth for women who worked in that interval. Opposite forces also operate: many women become trapped between accidental childbearing and financial pressure to work. The cummulative effect of growing family size is to depress labor force participation, but after two or three children the financial burden creates significant counter pressure favoring employment.

Significance to Biomedical Research and Program of the Institute: The study will contribute materially to the Center's program of understanding changes in fertility behavior of American women by allowing genuine causal analysis of the dramatic changes in fertility during this period.

Proposed Course: This study was initiated in FY 75, and the current series of analytical studies is expected to be completed during FY 81.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Impact of Childbearing and Spacing Patterns on the Health of the Mother and the Development of the Child in an Urban Population
Contract Number: NO1-HD-52835
Contractor : Brown University
Money Allocated: $138,073 (1975); $12,535 (1979)

Objectives: This study focused on the consequences of different childbearing and childrearing patterns at various ages. Data pertain to: (a) the health status of the mother for all 80,000 pregnancy events in Aberdeen, Scotland, 1948-1973; (b) age, marital status and socio-economic status of the mother and father; and (c) results of mental and physical tests administered to all 12,000 children born in Aberdeen, 1950-1954, through the age-11 school examination.

Major Findings: Pregnancy histories of 3100 once-married Aberdeen women show that fertility varied inversely by social class position among non-mobile women. Upwardly mobile women tended to postpone their first pregnancy outcome and to have lower fertility than other women in the same social class of origin. Higher status women are adopting the lower class practice of starting reproduction at younger ages. Moreover, younger women are more likely than older women to have begun reproduction prior to marriage. Social class in Scotland is the major variable accounting for differences in either physical or mental development; second is birth weight for physical development, and family size for mental development. Physical development at age 5 varies directly by social class--with increase in class status there is an increase in the proportion of taller and larger children. Mental development, as measured by the 7+, 9+, and 11+ tests, similarly increases directly by social status, but at each status level the average scores decrease with each increase in family size. Low birth weight has a negative impact on both physical and mental development. Large families, higher order births, low birth weight, low socio-economic status, and very young mothers all have a sizable and detrimental impact on both mental and physical development.

From 1953-1977 a substantial increase in the number of pregnancies among unmarried women under age 20 was accompanied by a nearly equal increase in induced abortions. The number of pregnancies among married women decreased markedly, and the number of births decreased even more so, due primarily to increased sterilizations. Infant deaths, stillbirths, and spontaneous abortions all decreased substantially, but the decreases were minor when compared with the very substantial increases in induced abortions. The net effect is that pregnancy wastage increased, e.g., with fewer than 75 percent of all pregnancies resulting in live births, 1973-1977.

Significance to Biomedical Research and Program of the Institute: The rich longitudinal data on the effects of childbearing and spacing patterns on the mother's health and the child's development are central to the Institute's concerns.

Proposed Course: This study is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Low Fertility Cohorts in the United States
Contract Number: NO1-HD-32799; NO1-HD-62818
Contractor : Georgetown University
Money Allocated: $99,911 (1973); $16,140 (1974); $22,913 (1975); $340,153 (1976); $424,410 (1977); $167,227 (1978); $70,516 (1979); $74,314 (1980)

Objectives: This study sought a deeper understanding of the low fertility of the 1920s and 1930s in the United States, by studying cohorts of women born during 1901-1910. The survey questionnaire included items on background characteristics, pregnancy and contraceptive history, attitudes toward family size, as well as measures of the influence of the Depression on the respondents' lives.

Major Findings: A majority of the women, 71 percent, reported that they or their husbands had used a contraceptive method. Of these respondents, 83 percent had used a method defined as modern (condom, diaphragm, jelly or cream, suppository or tablets, vaginal cap or IUD, contraceptive douche, or sterilization to prevent pregnancy), and 49 percent had initiated use within the first-birth interval. Analysis of the first-birth interval, which was limited to women with intact marriages throughout the reproductive period, showed that the median length of the interval was more than twice as long for contraceptive users (33 months) as for nonusers (14 months).

Further study of delay in childbearing within the first-birth interval showed a strong positive association between length of the interval (as well as contraceptive use and success in the interval) and wife's and husband's educations, husband's white-collar occupation, and the wife's working within the interval. Among fecund women with first marriages intact throughout the first-birth interval, the median length of the interval was 18.9 months; 39 percent of the women were contraceptive users, and 29 percent of the contraceptive users intended to remain childless.

Data from a subsample of 165 nonrespondents were analyzed to assess the extent of nonresponse bias in the Study of Low Fertility Cohorts. Results for the two groups were compared, and the differences were not statistically significant; thus, nonresponse did not seriously bias the data.

Significance to Biomedical Research and Program of the Institute: As part of the Institute's interest in understanding the determinants of fertility, this project fills a major research gap. The women studied had the lowest total fertility rates ever recorded in the United States, a phenomenon that occurred before the most efficient contraceptives became available. These women's motivations and the means they used to achieve record-low fertility are important to further understanding of the practice of birth control and the motivations for parenthood.

Proposed Course: This project is scheduled for completion in 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Alienation and Fertility Control in the Early Years of Marriage
Contract Number	:	NO1-HD-62833
Contractor	:	Bowling Green State University
Money Allocated	:	$142,361 (1976); $125,117 (1977); $111,309 (1978); $27,606 (1980)

Objectives: This research tested the relation between alienation and fertility with respect to a broad range of dependent variables, including family size preferences, early contraceptive and pregnancy decision-making, contraceptive use, premarital and unplanned pregnancies, and abortions. Interest centered on the early years of childbearing. Data were collected by means of self-administered questionnaires from 610 once-married couples, classified according to wife's age at marriage and marital duration. Methodologically, in addition to development of new measures of alienation, reversal of the alienation concepts resulted in positive measures of social integration. This work continued and expanded on previous work by the principal investigators.

Major Findings: The findings generally supported the hypothesis that kinds and degrees of alienation (feelings of normlessness, meaninglessness, and social isolation) interfere with rational family planning and fertility control. Subjects high in levels of alienation, compared with other subjects, tended to terminate their educations at lower levels, to marry at earlier ages, to be less knowledgeable about sex and reproduction, to use less effective contraceptive methods, but to see fewer advantages of having children.

Contraceptive use-effectiveness at first intercourse was associated with educational attainment, age at marriage, meaninglessness, and traditionalism. Current contraceptive risk-taking across varying levels of parity was also related, in the ways hypothesized, to meaninglessness, traditionalism, wife's plans for employment, and contraceptive use-effectiveness at first intercourse.

Seventy-eight percent of the couples (the wife, the husband, or both) reported plans for sterilization after achieving their desired family. Couples who agreed on having "no plans" for sterilization tended to have low levels of knowledge about sex and reproduction, high levels of traditionalism, and expected family sizes of three or more children. The data also showed a close correspondence between plans for sterilization and attitudes toward abortion. Couples who agreed on having plans for sterilization were likely to be tolerant of abortion, to be knowledgeable about sex and reproduction, and to expect small family sizes.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the antecedents and determinants of fertility.

Proposed Course: This study is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Fertility Values and Family Growth
Contract Number: NO1-HD-62834
Contractor : Temple University
Money Allocated: $180,753 (1976); $30,049 (1978)

Objectives: This project examines the developmental effects of actual fertility on fertility values within religious and SES subgroups. A previous study indicated that the experience of bearing children was related to people's perceptions of a range of values related to fertility and family-size desires. However, because longitudinal data were not available, it could not be determined whether people with three children had different values from those with no children because of the presence of children. The study proposed here will establish more clearly: (a) the effects of parity increase on the relationships of fertility values to desired family size; (b) the fertility value relationships that are associated with Catholic/Protestant affiliation, social class and those that are independent of these two factors; and (c) the parental experiences associated with an increase in parity.

Major Findings: Preliminary analyses of cross-sectional data produced a number of tentative conclusions which will be further tested when longitudinal data is available. Generally, the perceived costs of children are more related to family size desires than are the perceived benefits of children. Anticipation of the rewards of children were most important for the initiation of family building and recognition of costs of children were most important for the decision to terminate family growth only for upper status Protestants. The importance of the arrival of the first child in changing perceptions about childbearing is evident from two findings: (1) anticipated rewards of childbearing were found to be related to desires for larger families mainly for childless couples; (2) the proportion of respondents reporting unexpected problems when their (last) child arrived was significantly greater among women with only one child than among those with two children. However, the arrival and experience of each child in the family altered the relationships between fertility values and desired family size. The satisfaction of child care and development was an important fertility value only among Protestants. Concerns about family interactions in the form of giving attention among family members was an important fertility value, although its prominence varied among social sub-groups. Financial matters were a special fertility concern of the Protestants, particularly the lower status ones. Protestants differed from Catholics in their developmental pattern of expectations of and reactions to the consequences of children, but fertility values generally were little related to fertility desires among the Catholics.

Significance to Biomedical Research and Program of the Institute: This project will enhance our understanding of the process of family formation and improve our understanding of the consequences of family size by elaborating how fertility influences future decisions about having children.

Proposed Course: The first stage of this project was completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Effects of Adolescent Pregnancy on Mother-Infant Relationships: A Transactional Model
Contract Number : NO1-HD-62860
Contractor : George Peabody College for Teachers
Money Allocated : $138,264 (1977); $61,569 (1978)

Objectives: To analyze existing data in three studies evaluating the psychological and behavioral consequences (among socio-economically disadvantaged patients in Nashville General Hospital) related to early childbearing among pregnant adolescents in comparison with pregnant women above age 20.

Major Findings: This sample of newborns indicates significant relationships of age at menarche with maternal age in support of current theories of increased fecundity among younger women and implies that the younger pregnant adolescent may be biologically prepared for reproduction at an earlier age and is not necessarily more sexually active than her nonparent age mates. In conjunction with the findings of others, this suggests that menarchal age may be of more consequence than chronological age in adolescent fertility. No relationship was found between maternal age and measures of prenatal complications, obstetric performance and delivery outcome, suggesting that pregnant adolescents receiving adequate prenatal care are not at greater obstetric risk than older women. However, this research supports the hypothesis that younger, adolescent women pregnant for the first time experience more stress than do older women and women who have had several pregnancies. It also shows a significant relationship between the effects of anesthesia given to the mother predelivery and the behavior of the newborn, with a high dose given shortly before delivery resulting in the greatest behavioral decrements as measured by the Brazelton Behavioral Assessment Scale. Other studies have shown type and timing of medication to be related to parity and length of labor. Primiparous mothers are more likely to have more and stronger drugs administered. As the adolescent mother is more likely to be primiparous, the effect of drugs on the children of adolescents, operating through parity, may be considerable. Analyses also have shown that older mothers both talk to and look at their babies more often than do younger mothers. Increased talking and looking time were associated with higher scores on measures of motor and mental skills, with more positive maternal attitudes, such as closeness to the child, competence in meeting the baby's needs and encouragement of reciprocity, and with more positive perception of the baby's temperament. These findings suggest that interaction patterns play an important role in the social development of the child.

Significance to Biomedical Research and Program of the Institute: This project provides information on mothers at risk of mistreating their children and other information on the effects of prenatal training programs of use to the policymaker.

Proposed Course: This contract was completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Consequences of Late Adolescent Childbearing in the Early 1970s
Contract Number: NO1-HD-72819
Contractor : The Rand Corporation
Money Allocated: $120,700 (1977); $131,130 (1978); $46,600 (1979)

Objectives: This research examined the near-term consequences of late adolescent childbearing in five major areas of the young parents' lives: (1) dimensions of personality (self-esteem, locus of control, and adult role orientation); (2) future expectations and aspirations about work, education, and family growth; (3) women's perceptions and orientations about homemaking and work; (4) young parents' economic well-being; and (5) their economic dependency on their parents and society. Data were from the National Longitudinal Study (NLS), a random sample of about 22,000 seniors of the class of 1972 from 1200 high schools. Follow-up surveys were conducted in 1973, 1974, and 1976.

Major Findings: There is strong circumstantial evidence that a person who manages to avoid parenthood during adolescence will acquire more of the schooling, training, and other resources necessary to realize his or her full adult potential. The evidence from the NLS, however, suggests that the apparent linkage between early parenthood and later economic outcomes is, at least in some respects, tenuous.

For high school graduates, the effects of early marriage and parenthood on educational expectations were small relative to many other influential factors (ability, family background, etc.). Among married adolescents, parents differed little from nonparents in educational expectations when background characteristics and marriage dates were controlled. In short, young people with rather low educational expectations appeared to be self-selected into adolescent parenthood.

Young women's orientation toward work was closely related to their marital and parenthood status. The importance of careers to married women decreased near the marriage date and decreased further when parenthood was imminent. Married men, compared with unmarried men, attached somewhat more importance to their careers, but parenthood had only a slight effect on men's orientation toward work. Among married women, mothers seemed slightly less satisfied with their career progress than did non-mothers, and dissatisfaction seemed linked to the timing of parenthood. Although the pattern was similar among married men, the timing of parenthood was not important.

Significance to Biomedical Research and Program of the Institute: Adolescent fertility is of particular concern in the United States, since it accounts for 20 percent of all births. The consequences of adolescent fertility, assessed by this research, are thus a topic of high priority for the Institute.

Proposed Course: This project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : How Mother's Age and Circumstances Affect Children
Contract Number : NO1-HD-82806
Contractor : University of Southern California
Money Allocated : $95,058 (1978)

Objectives: This is a two-year study which makes use of sample data that were collected in 1963-65 in the Health Examination Survey conducted by NCHS in cooperation with the Bureau of the Census. The sample included children 6 to 11 years of age in a representative nationwide survey who were administered physical and psychological tests, whose parents and teachers provided background information and evaluations, whose records in the offices of their physicians, dentists, and hospitals were consulted, and whose birth certificates are to be obtained. This information is available for about 7000 children, 300 of whom can be identified as illegitimate. The purpose of the study is to determine whether youthful age of mother and growing up in a disrupted family adversely influences the development of the child, controlling for social class and birth order.

Major Findings: It has been found that there is an inverted U-shaped relationship between mother's age and the child's (10 and 11-year olds) intellectual and educational development. It may be inferred from the findings that the optimum age for a mother to have a child is between 23 and 29. In general, children's intellectual performance is lower if the mother is a teenager. However, when the IQ scores of the boys and girls were examined separately, both the Wechsler vocabulary and block design scores were lower for boys born to teenage mothers than for those born to older mothers. This relationship was not found for girls. When mother's and father's education, mother's marital status, race, income, and other relevant variables were controlled, no direct effect was found of mother's teenage status at time of birth on the child's likelihood of passing a grade in school. However, the combination of financial disadvantage or low parental education and teenage parenthood was found to be detrimental to the child's chances of passing grades. Mothers working fulltime, more educated mothers, and mothers who breastfeed have children who are less likely to fail in school.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and consequences of fertility.

Proposed Course: Contract expired this fiscal year.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	The Consequences of Family Structure and Maternal State for Child and Mother's Development
Contract Number	:	NO1-HD-82807
Contractor	:	University of Southern California
Money Allocated	:	$174,357 (1978); $98,849 (1979)

Objectives: This is a study of the effect of family size and childspacing upon the health and social development of children, using a Danish population of 9000 children born in 1959-61 in Copenhagen. Data will be analyzed to determine effects of order of birth and sex, age of mother, wantedness, spacing, perinatal factors, parental health and social and family structure variables. Additional data will be collected on development of children, school performance, delinquency, and psychological and social functioning.

Significance to Biomedical Research and Program of the Institute: This study explores the consequences of family size, birth order, and childspacing for a variety of health, psychological, and social outcomes.

Major Findings: In this study, a linear relationship was found between mother's age and pre-, peri-, and neo-natal problems, with teenage mothers experiencing the fewest complications. Similarly, no relationship was found between negative neonatal outcomes and social risk factors, such as low SES, illegitimacy, and unwantedness of pregnancy. These findings corroborate results of the American Collaborative Perinatal Study where University hospitals, which provided high quality health care, were also used. They contradict, however, the findings of most population studies involving more representative populations in the U.S., Britian and Denmark. The results were interpreted as an indication of the importance of adequate medical and health care, particularly for young and low SES mothers. Pregnancy complications, delivery complications, and birthweight were found to be the most significant predictors of infant survival and neonatal health. While low birthweight is the most important predictor of infant survival and health, none of the medical and social variables included in the study predicted birthweight.

Neurological functioning and motor development at one year of age appear to be associated with medical and biological factors. However, physical health at one year is related to social factors. Children of low SES and children of unmarried mothers who live alone with their children generally show poorer physical health. The study also finds that children of older mothers, 25 years and up, do better than children of younger mothers, except in cases where teenage mothers rely upon older women (i.e., grandmothers) for child care. In a 12-year follow-up study of lowbirth weight infants, higher incidences of mental retardation and behavior disorders were found among the low birthweight children. The data also suggested that socio-economic status is very important in enabling an infant to recover from perinatal trauma.

Proposed Course: This study is expected to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Psychological Factors and Fertility Regulation in Women: Data Analysis
Contract Number: NO1-HD-82831
Contractor : American Institutes for Research, Palo Alto, California
Money Allocated: $110,569 (1978)

Objectives: This study analyzed extant data collected in a study of psychological and behavioral factors affecting fertility regulation in women. Data were gathered annually over a three year period for three main subject groups: 325 never-married women, 322 just-married women, and 320 just mothered women (with first child). The study sample was of restricted age and marital status, all fecund, white, and English-speaking women, selected randomly from a specified urban/suburban area around Stanford, California. Semi-structured interviews and self-report questionaires focused on psychological and behavioral aspects of (a) cohabitation, marriage, sexuality, contraception, gender role activities, occupational activities, and divorce as each of these domains affected fertility, and on (b) proception, childbearing, and childrearing.

Major Findings: Findings show that the biological capacity to conceive, proception (behavior intended to achieve conception) and contraception are the primary determinants of conception among married women. Proception depends upon the woman's own psychology, the opinions of significant others and situational factors including parity. Successful contraception depends upon the type of method used and on how regularly it is used. More than two-thirds of the conceptions within marriage are fully intended and about three-quarters are actively wanted. Around six percent are terminated with an induced abortion. The pattern for non-marital conceptions is very different, with most being unintended and rejected. There are seven aspects of couple interaction relevant to reproduction: communication, conflict, influence, decision-making, bonding, division of labor, and adaptation to change. The first four directly affect the psychological sequence leading to having a child. Bonding affects marriage and marital dissolution which, in turn, respectively facilitate and inhibit childbearing. The division of labor is reflected in female role performance and female role orientation. The latter also affects the psychological sequence that leads to having a child. It is clear that reproduction-related events such as the birth of a child, an induced abortion, marriage, and marital dissolution have important effects on the woman and her childbearing expectations. Employment status has only a small effect on the woman's childbearing expectations and behavior. On the other hand, the woman's childbearing expectations and the number of children she has at home have a substantial effect on her work status.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the correlates and determinants of fertility regulation behavior.

Proposed Course: This contract expired December 31, 1979.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : American Attitudes Toward Abortion
Contract Number : NO1-HD-82832
Contractor : University of California at Los Angeles
Money Allocated : $92,668 (1978); $13,725 (1980)

Objectives: This study analyzes existing data and generates new data on abortion attitudes over a three-year period as expressed in U.S. national samples interviewed by the Gallup Poll. The emphasis is on demographic and social-psychological correlates of attitudes toward various aspects of the abortion issue. The two phases are interrelated. The first emphasizes descriptive, socio-demographic and locational aspects of abortion attitudes, including changes over time. The second major component studies sociological and personality predictors of abortion attitudes. The project utilizes Gallup survey data going back to 1962, new data to be collected during the course of the contract, data from the National Opinion Research Center (NORC) surveys, and information from the 1965 and 1970 National Fertility Surveys (NFS). Disaggregation of abortion attitudes as the dependent variable--timing of abortion, belief about when life or personhood begins, government aid for abortion, etc.--appears to be increasingly significant as people become more aware of the complexity of the abortion problem.

Major Findings: An analysis of previous survey questions on the conditions under which people regard induced abortion as justified (mother's health, birth deformity, economic stress, and elective abortion) reveals that the most important distinction is between whether people disapprove abortion for all reasons or approve for all reasons and not whether they express approval or disapproval according to graded justifications. However, analysis of the characteristics of the equivocators, the group approving of abortion under some conditions, indicates that they are very similar to each other in their background characteristics and are more similar to those who are altogether negative about abortion than to those who are positive. By analyzing many surveys over time it is possible to see that the characteristics of the equivocators have become slightly more like those who approve of abortion for all reasons. The study of equivocators is becoming increasingly important as a recent rise in the proportion of equivocators has brought that position to an all-time high. The investigator is also finding what appears to be the development of more clear-cut ideological differences among people belonging to the various Protestant denominations. In some cases, these differences appear to be even greater than the Catholic- non-Catholic differences in views toward induced abortion.

Significance to Biomedical Research and Program of the Institute: This research, is central to the Program of the Center and is highly relevant to an understanding of the determinants of choice among various methods of fertility control.

Proposed Course: Additional surveys will be conducted over the next two years and the project is scheduled to be completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Psychological Factors Associated with Fertility Regulation: A Comparison of Pregnant and Non-Pregnant Adolescents
Contract Number : NO1-HD-82833
Contractor : The Philadelphia Health Management Corp., Philadelphia, Pa.
Money Allocated : $85,585 (1978); $115,055 (1979); $61,499 (1980)

Objectives: The primary purpose of this study is to examine the psychological/thought processes operating when a female adolescent does or does not become pregnant. A secondary purpose is to conduct an exploratory study of male adolescents' knowledge and attitudes toward contraception and attitudes toward teenage fatherhood.

The subjects for this study are predominantly black female and male: urban adolescents of low socioeconomic status (SES). The first sample consists of 90 adolescents in the third trimester of pregnancy who have volunteered for one of two alternative programs run by the Philadelphia School District; these adolescents will be interviewed once, as they enter the program. The second sample is a comparison group of 180 non-pregnant adolescents, matched (2 to 1) with the first group on age, residential location (as an indicator of SES), and race. These adolescents will be interviewed twice. At the initial interview they will not be pregnant; they will be interviewed a year later, when it is expected that approxmately 25 percent will have become pregnant. The third sample consists of 90 male black adolescents who have volunteered for a school district group discussion series on sexuality.

Major Findings: Preliminary analysis of the 132 interviews conducted thus far with female adolescents has shown significant differences in educational aspirations, plans for motherhood, and problem solving skills among four categories of young women: pregnant adolescents, sexually active noncontraceptors, sexually active contraceptors, and virgins. Pregnant adolescents are much less likely than others to want to attend college and are more likely to have fallen behind in school, even before becoming pregnant. Noncontraceptors want their first child at a younger age than contraceptors or virgins; 15 percent of the noncontraceptors want their first child at age 17 or 18. Contraceptors show more ability than noncontraceptors or pregnant adolescents to plan for personal goals and to find alternative solutions for personal problems. Other findings indicate that daughters of employed mothers are more likely than daughters of housewives to know about birth control and the negative consequences of pregnancy, to have higher educational aspirations, and to use contraceptives. Mothers with low aspirations for their daughters' educations tend to have daughters who do not practice contraception.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the correlates of adolescent fertility behavior.

Proposed Course: This project is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	A Multifaceted Analysis of Abortion as a Social Issue in the United States, 1965-1978
Contract Number	:	NO1-HD-82834
Contractor	:	University of Missouri
Money Allocated	:	$45,714 (1978); $19,539 (1980)

Objectives: The purpose is to analyze abortion attitudes in the U.S. between 1965 and 1978. Available national survey data are being used to describe the climate of opinion and changes over time and to integrate the findings so as to enhance the understanding of psycho-social issues underlying abortion attitudes. Among the variables being considered in relation to abortion attitudes are socio-economic indicators including education, political ideology, religion and views on women's liberation and human rights.

Major Findings: Attitudes toward making legal abortion available became much more favorable between 1965 and 1973 and did not change until 1978, when there was a small but significant decrease in approval of abortion for social-psychological reasons. Education was the socio-economic status indicator most strongly correlated to abortion attitudes, with approval of abortion increasing with the amount of formal education. Jews and those without religious affiliations were the groups most approving of legal abortion. Among Protestants, Episcopalians were the most approving, and Baptists the least approving. Catholics were consistently more opposed than Protestants. The Protestant-Catholic difference was found to increase as education increased, that is, opposition to abortion decreased with an increase in education for Protestants but not for Catholics. Approval of legalized abortion decreased slightly as age increased and was slightly higher among whites than blacks. People in urban and suburban settings were more approving of legalized abortion than people in rural areas. A slight tendency was found for political conservatism to be correlated with disapproval of legal abortion, especially among people with some college education. Abortion attitudes were consistently and strongly linked to conservatism regarding matters of personal morality. Those disapproving of abortion also disapproved of euthanasia and suicide and preferred large families.

Significance to Biomedical Research and Program of the Institute: Each year over one million women terminate pregnancies by means of a legal abortion. This research addresses factors relating to this major health issue.

Proposed Course: This project began in FY 78.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Proposal to Investigate the Determinants of Variations in U.S. Fertility Trends 1940-1975
Contract Number: NO1-HD-82835
Contractor : Mathematica Policy Research, Inc.
Money Allocated: $102,443 (1978); $42,356 (1979)

Objectives: This research examined trends in fertility (both the timing of births and completed fertility) and female labor-force participation, vis-a-vis male relative income, female wage rates, and government income transfer payments from 1940-1975. Relative income was a measure designed to reflect the earnings potential of young men, relative to their aspirations. Transfer income had three components: Old Age and Survivors' Insurance, Unemployment Insurance, and Aid to Families with Dependent Children.

Major Findings: The research results suggested that male relative income and female wage rates had a strong influence on fertility and female labor-force participation. Higher relative income appeared to increase completed fertility and to decrease female labor-force participation. In addition, higher relative income appeared to induce both earlier childbearing and earlier female labor-force participation. The findings suggested, however, that female wage rates were the most important factor in recent fertility trends and female labor supply. Increased female wage rates, mainly during the past 20 years, were associated with lower completed fertility, longer postponement of childbearing, and increased female labor-force participation.

When transfer payments were analyzed with aggregate data for 1947-1975, it was not possible to separate the effect of transfer income from that of other income on fertility and female labor-force participation. However, another analysis, using data from a longitudinal survey of young women during 1968-1975, failed to show any significant effect of transfer payments on fertility.

Significance to Biomedical Research and Program of the Institute: This research, dealing with certain factors that have contributed to wide fluctuations in fertility since the 1940s, is relevant to the program of the Institute. Moreover, the research findings provide an importnat insight into public policy, since they suggest that government income transfer payments have not affected fertility.

Proposed Course: This project began in 1978 and is scheduled for completion in 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Adolescents' Use of Information about Conception and Contraception
Contract Number	:	NO1-HD-82836
Contractor	:	Planned Parenthood Association/Chicago
Money Allocated	:	$91,224 (1978); $61,419 (1979)

Objectives: Couples of different ethnicities from five Chicago area teen family planning clinics were chosen by quotas within each clinic to obtain five females seeking contraception, five who are pregnant, and five with a negative pregnancy test or "pregnancy scare." At the end of one year, 50 additional female adolescents will be interviewed as controls. Piaget's theoretical work suggests that mere assessment of knowledge levels among adolescents concerning contraception and other sexual matters is insufficient to understand the role of that knowledge in behavior , i.e., that adolescents assimilate knowledge selectively and then use it differently depending on their own situations and cognitive processes.

Major Findings: Preliminary results from focused interviews with the 75 adolescent females and their male partners indicate that despite exposure to presentations about contraceptive methods, reproductive anatomy and physiology, many internalize very little information and experience difficulties in accepting and using available methods. Adolescents, encouraged to manipulate and ask questions about the various methods, demonstrated with plastic models and anatomical diagrams, accept but little remember them. The translation from relatively abstract presentations to the young women's own bodily processes does not occur, for their minds are occupied with worries about pregnancy, pelvic examinations, and a variety of serious concerns about other aspects of their lives. Male partners, even less knowledgeable, focus attention almost entirely on other matters, except for occasional concerns about pregnancy. Even before selecting a method of contraception, adolescent partners anticipate problems with using any method. Many make serious mistakes in their use of contraceptive methods; others use methods correctly but fear that they are making mistakes. Proper use is influenced by the availability of clinic appointments, the sensitivity of clinic personnel to adolescents' concerns about methods, and the support and encouragement of male partners and parents.

Significance to Biomedical Research and Program of the Institute: This research is central to the Institute's program and is highly relevant to departmental initiatives and to Congressional interest in the effectiveness of present modes of presenting such information to adolescents.

Proposed Course: This project is scheduled to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Adolescents' Perspectives on the Health Care System: A Determinant of Fertility
Contract Number : NO1-HD-82837
Contractor : University of North Carolina
Money Allocated : $105,786 (1978); $108,120 (1979); $58,225 (1980)

Objectives: The purpose of this research is to identify the nature and sources of adolescent women's perspectives on the established medical care system, and the impact of these perspectives on their use of the system for fertility-related services. The specific objectives are: to identify the reasons why adolescent women may be reluctant to use the established medical care system for fertility-related services, and to assess the usefulness of various techniques for collecting data about adolescents.

Several methods will be used to obtain a comprehensive list of adolescents' concerns regarding medical care. A typology will be developed along with a conceptualization that is empirically verifiable. The research will involve open-ended group and individual interviews, in formats determined by a Research Advisory Panel of adolescents, to obtain information from 200-300 adolescent women. Findings will provide bases for further research and guidelines for improving medical services for adolescent women.

Major Findings: Preliminary analysis of open-ended group interviews has identified several recurrent themes. In choosing a physician, adolescents are constrained by cost; fear that information will not be kept confidential; and anticipated lack of privacy during the physical examination. Concerns about communicating with physicians are that they do not consider adolescents as people with feelings and rights; give inadequate explanations; are judgmental; and ask excessively personal questions. Adolescents characterize as ideal a physician who encourages questions, gives good explanations, and helps the adolescent to relax; is neither too old nor too young; is female; is friendly and sincere. Problems with parents that affect adolescents' reproductive health care include the need to have information about their sexual activity kept confidential from parents; need for parents to make their medical appointments; and need for financial support from parents. Adolescents identified as issues in reproductive health care the need for more and better sex education; fear of pregnancy and venereal diseases; and embarrassment and guilt about sexual activity, feelings that are evoked by encounters with physicians.

Significance to Biomedical Research and Program of the Institute: Other studies have pointed to the high proportion of adolescent contraceptors who use clinic services, but little research has addressed adolescents' avoidance of the established medical care system. This system may be the only one large enough to meet adolescents' demands for family planning and related services.

Proposed course: The project was begun in FY 1978 and is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Research on the Determinants of Repeated Adolescent Pregnancy and Childbearing
Contract Number	:	NO1-HD-82838
Contractor	:	Johns Hopkins University
Money Allocated	:	$52,752 (1978)

Objectives: This project used the extant data base of a Johns Hopkins longitudinal research and service program established in 1973 for adolescent mothers to identify those young women who had a subsequent (unwanted) pregnancy and to compare them with matched controls who did not experience a subsequent pregnancy. The study, which included about 90 cases and an equivalent number of controls matched by race and age, analyzed data collected during the first pregnancy and at the four-week postpartum visit. The data available about mother, father, and pregnancy included; demographic, educational, and family background variables; contraceptive use; amount of prenatal education; and contraceptive prescribed postpartum. These objectives were to provide: a broad and complete picture of the problem among these urban adolescents; gross indicators of risk of repeated pregnancy; and information about the outcome of subsequent pregnancy.

Major Findings: Preliminary findings showed that adolescents who became pregnant again, compared with those who did not, were more likely to be married, younger, welfare recipients, less intellectually competent; to have had a prior pregnancy terminated by abortion; to have worked less; and to have had more problems using contraceptives. Of the first 40 repeat pregnancies studied, all but seven were terminated by elective abortion; most of the seven babies carried to term were unplanned and undesired.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing," is central to the program of the Institute and is highly relevant to Congressional interests. The project's considerable value lies in the study of young people who create hardships for themselves and society--adolescents with repeat pregnancies. The research may generate predictive data about individual characteristics as well as identify the structural sources of repeat pregnancies.

Proposed Course: This project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sociosexual Development in Black Adolescents within the Family Context: A Longitudinal Study
Contract Number: NO1-HD-82840
Contractor : Howard University Hospital
Money Allocated: $99,499 (1978); $107,253 (1979); $112,630 (1980)

Objectives: The purpose is to explore the effects of psychological adjustment, sexual knowledge and attitudes, family interaction, moral and ethical reasoning, socio-economic status and environmental impact on sexual behavior. The project involves a five-year longitudinal study of 160 black early adolescents and their parents. Periodic medical examinations, unstructured interviews using a variety of stimulus materials, and videotaping of family interaction will be used. Participants will be a volunteer non-responsive sample of healthy 10-year-olds from low and middle income families, evenly split male and female. Test materials (pictures, incomplete stories and simulated taped conversations) will be developed. Some family interaction observations will be made.

Major Findings: The following observations represent trends rather than statistically established relationships, since the total participant group has not yet been examined. The participants could be divided into three groups according to their level of heterosexual interest and physical involvement. Children did not differ markedly regarding same sex interest. Masturbation was admitted to by only two participants. The three groups are (1) those preadolescents with some early heterosexual experience involving touching; (2) those preadolescents describing a boyfriend/girlfriend relationship with no heterosexual experience involving touching; and (3) those preadolescents with no boyfriend/girlfriend relationship or heterosexual experience. The early trend appears to be that there is very little difference among these groups in previous experimentation with beer, wine, or cigarettes, and no difference in acquaintance with marijuana use. The sex of the participants did not vary markedly among the three groups nor did the stage of pubertal development. The income level and the WRAT percentile (greater or lower than 50th percentile) did differ with the preadolescents with no experience, no boyfriend/girlfriend, being predominantly middle income and with higher acheivement scores than the other two groups. Income level and achievement scores had a very strong direct relationship.

Significance to Biomedical Research and Program of the Institute: This study addresses the social and physiological changes around adolescence relating to a sexual development. Concern about adolescent sexual and reproductive behavior is shared by biomedical and social scientists. This proposal addresses a critical juncture of the two fields on an issue of considerable importance.

Proposed Course: This project began in FY 1978 and is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Analysis of the Determinants of Adolescent Pregnancy and Childbearing
Contract Number: NO1-HD-82841
Contractor : Mathematica Policy Research, Inc.
Money Allocated: $83,695 (1978); $49,120 (1979)

Objectives: Data from the 1971 Kantner and Zelnick national probability sample of females aged 15-19 were used to analyze three components of adolescent fertility behavior: sexual activity, contraceptive practice, and pregnancy. Preliminary analysis of a fourth component, decisions about the outcome of pregnancy, is in progress. The three components were examined in relation to pertinent socioeconomic variables: labor market experience, family structure and background, education, and sexual and contraceptive knowledge. The research was undertaken to develop a model of adolescent fertility behavior that would incorporate these socioeconomic variables.

Major Findings: Educational expectations were negatively associated with the probability of intercourse; however, in the subsample of sexually active women, educational attainment and expectations were not related to contraceptive practice or the incidence of pregnancy. Women who worked and attended school were less likely to be sexually active, but those who were sexually active were less likely to use contraceptives. Moreover, working without attending school was associated with a higher probability of intercourse.

Sexual and contraceptive knowledge showed the strongest association with fertility behavior. Women who knew when they were likely to become pregnant and knew about medical methods of contraception were more likely to be sexually active and to use contraceptives. However, it was not possible to ascertain whether knowledge preceded sexual activity and contraceptive practice, or vice versa.

Family background was strongly associated with the probability of pregnancy and also was related to sexual activity and contraceptive practice. Parents' educational attainment and family income were negatively related to the probability of pregnancy.

The results summarized above refer to the entire sample. A separate analysis showed significant racial differences in the probabilities of intercourse, contraceptive practice, and pregnancy.

Significance to Biomedical Research and Program of the Institute: Adolescent pregnancy is a problem of high priority for the Institute. This research on fertility behavior pertains directly to the antecedents and prevention of adolescent pregnancy.

Proposed Course: This project began in 1978 and is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Contraceptive Use-Effectiveness: The Fit Between User and Method Characteristics
Contract Number	:	NO1-HD-82842
Contractor	:	Claremont Graduate School
Money Allocated	:	$49,492 (1979)

Objectives: An intensive archival study of current and past contraceptive clinic records investigated the use-effectiveness of various contraceptive methods for specific subgroups of the adolescent clinic population, to assess the relation between method characteristics and user characteristics in determining success or failure in contraception. The data were collected from 800 adolescent contraceptors, who were given extensive personality tests, knowledge-attitude, and practices questionnaires, and interviews at clinic intake, and were followed for three years. One-seventh became pregnant; the rest dropped out, changed methods, or had various other contraceptive use-effectiveness outcomes. Use-effectiveness outcome variables other than unwanted pregnancy were related to earlier personality, knowledge, and attitude measures to determine the degree to which prior knowledge, attitudes, and personal characteristics influenced later use-effectiveness of contraception.

Major Findings: The research results confirmed hypotheses relating personal characteristics to contraceptive side effects, especially side effects that were judged to have led to failure in contraception. Specifically, adolescents initially assessed as: (1) reporting more negative family relationships earlier in life; (2) showing lower levels of socialization and generally displaying more socially unacceptable behavior; (3) having less ability to think ahead into the future; (4) having a poorer self-concept; and (5) displaying other negative personal characteristics experienced more side effects during the program of contraception, and more side effects that led to failure in contraception. Also, medical problems generally were related to personal characteristics. Demographic characteristics such as age and ethnic background were found not to be related systematically to contraceptive side effects.

These findings suggest a role for psychological determinants of what are usually considered physiological problems in contraception. The study results have important implications for the prediction and prevention of contraceptive failure and have additional relevance for health care and public policy.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing," is central to the program of the Institute and is highly relevant to departmental initiatives and to Congressional interests. The findings have direct and immediate applications at the service delivery level and should promise valuable information about a little-known area.

Proposed Course: This project is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Policy Determinants of Teenage Childbearing
Contract Number : NO1-HD-82843
Contractor : The Urban Institute
Money Allocated : $64,253 (1978); $30,657 (1979)

Objectives: This research assessed the relations of various state and federal programs to adolescent childbearing. The programs presumed to have a direct effect were family planning services, abortion availability, age of consent laws concerning contraception and abortion, Medicaid coverage of abortion, welfare benefit levels and acceptance rates, welfare coverage of unborn children and unemployed fathers, and benefit levels in food stamp and Medicaid programs. Indirect policy influences included local unemployment rates, female wages, and educational opportunity. State information on these programs and policies was added to the 1976 public use tape of the Survey of Income and Education. This survey provided data on fertility, demographic, and family characteristics of an estimated 30,000 females aged 14-19. Two sets of analyses were performed: (1) characteristics of individual females, plus state-level policy and control variables, were regressed on whether a female aged 14-19 had a child under one year of age; and (2) state-level policy variables plus state-level control variables were regressed on adolescent fertility rates in each state and a subset of cities.

Major Findings: The research results did not substantiate the hypothesized influence of state and federal programs on adolescent childbearing. In fact, measures of benefits from Aid to Families with Dependent Children (AFDC), when statistically significant, were consistently negative, and AFDC policy variables were consistently unimportant. Similarly, most measures of abortion availability were not significantly related to the dependent variable; however, the several significant measures suggested that greater availability was associated with lower fertility. Most family planning measures were not significant, and the significant measures varied in direction. Measures of educational opportunity did show a fairly consistent relation between higher female educational achievement and lower adolescent fertility. In this analysis the availability of sex education did not affect fertility, but a more refined measure of availability is needed. The research found weak evidence that employment opportunity was negatively related to adolescent fertility. In the state-level analysis the employment rate was negatively related to fertility, but the median income of women employed full-time was positively related. Other measures of employment opportunity tended to be negatively associated with the dependent variable, though often the associations were not statistically significant.

Significance to Biomedical Research and Program of the Institute: This research addresses a topic of considerable importance and interest in both biomedical and social science areas--adolescent fertility.

Proposed Course: This project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Exploration of the Effects of Organized Family Planning Programs in the United States on Adolescent Fertility
Contract Number: NO1-HD-82844
Contractor : The Alan Guttmacher Institute
Money Allocated: $74,546 (1978); $56,601 (1979)

Objectives: This study assessed the impact of family planning clinic programs in the United States on the fertility of black and white female adolescents (aged 15-19). Several measures of social, demographic and family planning service characteristics were compiled for Statistical Analysis Units (counties or clusters of counties) and then subjected to multivariate analysis to determine: (1) the association between adolescent birth rates and the availablility and utilization of family planning clinic services; and (2) the association between changes in the availability and utilization of clinic services and changes in adolescent fertility rates from 1970-1976. The multivariate analysis controlled other determinants of adolescent birth rates and of the impact of abortion and private physician services as alternatives to clinic services. Data sources included: (1) county-level data from special tabulations of the 1970 Census to provide 1970 fertility rates for women aged 15-19 by race, as well as family planning program data for 1969 and socioeconomic and demographic control variables for 1970; and (2) newly available data for 1975 and 1976.

Major Findings: Preliminary multiple regression analysis showed that on a cross-sectional basis (1970 and 1975), areas with higher proportions of adolescents enrolled in family planning programs were also areas with higher adolescent birth rates. This is a common finding in many countries, due to the location of clinics in high fertility areas and to differences in socioeconomic characteristics and levels of sexual activity.

As expected, early findings showed that areas with greater increases in the proportion of adolescents enrolled in family planning clinics between 1970 and 1975 generally had greater decreases in adolescent birth rates. Analysis to understand the factors behind the cross-sectional pattern and the change over-time is continuing. Pronounced differences by region (Northeast, North Central, South, and West) and by race are now being explored.

Significance to Biomedical Research and Program of the Institute: This research is central to the program of the Institute. Though essentially an evaluation of family planning clinic services to adolescents, and thus highly focused on one determinant of adolescent fertility, the study addresses an important policy issue: whether differential availability of and enrollment in organized family planning programs measurably affects adolescent fertility. The study design should provide indirect evidence of this effect by presenting areal or ecological relations between the variables.

Proposed Course: This project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Psychosocial Determinants of Adolescent Contraceptive Use
Contract Number : NO1-HD-82847
Contractor : The George Washington University
Money Allocated : $31,218 (1978)

Objectives: This research sought to identify the psychosocial determinants of adolescent sexual activity (petting, intercourse, frequency of intercourse, number of partners); contraceptive use (unprotected intercourse, frequency of contraception); and pregnancy. The psychosocial factors were personality; attitudes about family roles, sex, and contraception; social experiences and expectations; and social pressures. Data were from an American Public Health Association survey of 831 16- and 17-year-old males and females. Also analyzed was a 30-month longitudinal information from a subsample of about 200 of the original respondents. The longitudinal sample was used to trace causal relations between psychosocial factors and fertility-related behavior. Demographic and social-class data were examined for their mediating effects on the three dependent variables.

Major Findings: The psychosocial factors predicted sexual behavior better than contraceptive behavior or pregnancy. Although many psychosocial factors covaried significantly with sexual and contraceptive behavior, most of these could not be classified definitively as antecedent or consequent to the behavior. The factors that could be so classified were usually consequences not antecedents. The most predictive psychosocial factors were attitudes that directly corresponded to the behavior. Contemporary attitudes about sex, especially approval of sexual activity for adolescents in love, most strongly predicted sexual behavior. However, this approval seemed to be a consequent rather than an antecedent of sexual behavior. Willingness to have unprotected intercourse was the strongest predictor of contraceptive nonuse; but other attitudes about contraception, especially perceptions of barriers to its use, also were predictive. In this analysis very few psychosocial factors differentiated female adolescents who had ever been pregnant from those never pregnant.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing," is central to the Institute's program. The research examined little-studied factors in fertility behavior, for example, the relation between perceived lack of access to contraceptives and nonuse of contraceptives. Two research results, longitudinal assessment of causal relations and parallel analyses of male and female adolescents, make this project particularly valuable.

Proposed Course: This project was completed in November 1979.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Determinants of Fertility Behavior Among U.S. Females Aged 15-19, 1971 and 1976
Contract Number : NO1-HD-82848
Contractor : The Johns Hopkins University
Money Allocated : $109,576 (1978); $116,951 (1979)

Objectives: The researchers analyzed adolescent sexual, contraceptive, and reproductive behavior vis-a-vis clusters of independent variables: family background; socioeconomic status (SES); relative influence of parents and peers; religion; residence; plans for marriage, childbearing, education, and career; perception of norms about illegitimacy and abortion; employment; social life; and dating behavior. Data were from two national surveys of women aged 15-19, conducted in 1971 and 1976 under a grant from NICHD. The research methods included cross-tabulations, with race controlled and multivariate analysis.

Major Findings: The findings showed increased but not extensive use of effective contraception by unmarried adolescent females. The major reason given for non-use of contraception was the belief that pregnancy could not occur, and another important reason was that intercourse was unexpected. About 20 percent of the women had intercourse by age 16 and 66 percent by age 18. Pregnancy was fairly widespread among women sexually active before marriage; 25 percent became pregnant by age 17 and more than 33 percent by age 19.

The research considered four aspects of premarital intercourse: prevalence, mean age at first intercourse, and number of premarital sexual partners for both never-married and ever-married women; and frequency of intercourse for never-married women only. Premarital intercourse was more prevalent among blacks, older adolescents, and women of low SES and religiosity. Women reared by their natural parents were less likely to be sexually experienced than were women reared in other family situations. Among sexually experienced women, age at first intercourse was lower for blacks, women of lower SES and religiosity, non-Catholics, and women not reared by their natural parents. Age at menarche was directly related to age at first intercourse and inversely related to prevalence of premarital intercourse. Whites had more premarital sexual partners than blacks, and the longer a woman was sexually active, the more partners she had. Plans to marry, use of medical contraceptive methods, and number of premarital sexual partners were directly associated with frequency of intercourse; frequency was higher for whites than blacks.

Significance to Biomedical Research and Program of the Institute: This research on adolescent fertility behavior addresses a topic of high priority to the Institute, using a major data source for the United States.

Proposed Course: This two-year project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Social and Cognitive Development of Only and First-Born Children
Contract Number	:	NO1-HD-82849
Contractor	:	Educational Testing Service, Princeton, New Jersey
Money Allocated	:	$92,576 (1978); $95,844 (1979)

Objectives: The research used data from a longitudinal study of children, their mothers, and mother-child interaction to make longitudinal and cross-sectional analyses contrasting the social behavior and cognitive development of only and first-born children at ages 3, 12, 24, and 36 months. Planned and unplanned children also were compared. The decision whether or not to have a second child was examined from the perspective of the mother's locus of control, femininity, concept of ideal family size, and quality of interaction with the first child.

Major Findings: Preliminary findings showed that only children whose parents planned to have additional births performed consistently higher on tests of cognitive functioning than did other only children. However, the birth of siblings adversely affected the cognitive performance of first-born children aged 2 years or less.

Different social behavior by only and first-born children was most evident at age 3 months, when only children cried more and smiled less. At ages 12 and 24 months, virtually no differences were found. It is uncertain whether the initial contrast was due to temperament or to mother-infant interaction. Although the percentage of abnormal pregnancies and deliveries was slightly higher for mothers of only children, these mothers also were more responsive to their 3-month-old infants. In other words, there was an appropriate match between fussy infants and attentive, soothing mothers.

Several factors increased the probability that an only child would remain without siblings for at least the first 40 months of life: a parent's belief that a small family was ideal, a difficult pregnancy and delivery, much crying and little smiling by the child, and a mother's perception that her child needed much attention.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the determinants and consequences of fertility behavior. More specifically, the findings represent a needed contribution to objective information about only children.

Proposed Course: This project began in FY 1978 and is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	The Consequences of Remaining Childless or Having Only One Child: An Analysis of Selected Outcomes for Husbands and Wives
Contract Number	:	NO1-HD-82850
Contractor	:	The Urban Institute
Money Allocated	:	$88,267 (1978); $89,107 (1979)

Objectives: This study analyzed the consequences of family size, specifically, zero and one-child families in relation to families with more children. The issues addressed were: economic well-being; time availability; schooling, training and labor force experience; geographic mobility; marital stability; and health. Besides the particular number of children, a concern of this research was the timing of births within the marital cycle. The analysis was based on the Michigan Panel Study of Income Dynamics, which studied heads of families, spouses, and their families from 1968-1976.

Major Findings: Differences in living standards for families with no children, one child, or two children were very small. Not until families had four or more children were disparities evident. Birth spacing, but not timing, also affected living standards. Consumption patterns of childless and small families differed somewhat from those of large families. Differences in per-capita food expenditures and in crowding were found, but they were slight. One- and two-child families were more likely to hire household help than were either large or childless families, and small families were more likely than large ones to have children in day-care centers.

Wives with children were more likely than childless wives to obtain additional training or schooling; however, they had less work experience and, early in marriage, slightly lower wages. Children depressed their parents' geographic mobility, and the presence of young children decreased the probability of their parents' divorce or separation. Having many children did not appear related to poor parental health.

Families of varying sizes differed primarily in their use of time. Time spent in paid work and in housework increased greatly as the number of children increased, and leisure time decreased accordingly.

Significance to Biomedical Research and Program of the Institute: Because increasing numbers of couples are electing not to have children or to have only one child, the consequences of such behavior are of growing interest and concern. This study assessed both the social and health consequences of very low fertility.

Proposed Course: This project began in FY 1978 and is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Studies on the One-Child Family
Contract Number : NO1-HD-82851
Contractor : University of California, Berkeley
Money Allocated : $91,452 (1978); $69,592 (1979)

Objectives: This project compared only children and children with siblings on various physical and behavioral measures. Data, consisting of records of children enrolled in the Kaiser Health Plan, were collected during the mother's pregnancy and when the child was 5, 10, and 15-17 years old. The records included information on the mother's reaction to the pregnancy; the child's physical growth, cognitive abilities, and behavioral characteristics; and prospective data on intentions to have future children and contraceptive use. Consequences of being an only child (compared to being oldest and youngest in two-and three-child families) were analyzed, and the determinants of the one-child family were described.

Major Findings: One-child families apparently were rarely the result of deliberate choice. Late marriage or marital disruption after the birth of the first child had occurred in about half of the single-child families. Only 5 percent of the mothers who had a 5-year-old only child reported having planned for this outcome. One-child families, compared to those with two or three children, showed no significant differences at the time of the oldest child's birth in mother's or father's education, father's occupation, family income, mother's work history, or mother's occupation. The mother of an only child was no more likely than other mothers to have furthered her education, but she was more likely to have taken work outside the home, to have experienced marital disruption and/or remarriage, and to have relied on a child-care service or nursery school.

The research showed no adverse effects of being an only child. There were no important differences between only children and the eldest of two or three children in physical development, utilization of medical care, or behavioral ratings; and there was some evidence that only children had marginally superior cognitive abilities.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the determinants and consequences of fertility behavior. More specifically, the findings represent a needed contribution to objective information about only children.

Proposed Course: This project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The One-Parent/One Child Family: Sociological and Psychological Consequences
Contract No. : NO1-HD-82852
Contractor : American Institutes for Research in the Behavioral Sciences
Money Allocated: $47,235 (1978) $71,496 (1979)

Objectives: The aim of this study is to make an exploratory examination of the structure, functioning, and interpersonal dynamics of a family form that includes a mother and child in a two-person family and compares this family form with three other family forms: the two parent/one child family; the one-parent/two child family and the one-parent/three child family. The families in the study shall be divided among those with children from age six or under to those with children up to 18 years old. Clinical, exploratory interviews will be conducted with 100 women and with their children (in the adolescent subsample). The focus of the study will be on the mother in terms of her work, leisure, economic well-being, educational pursuits, and perceptions of parental role, and on the child in terms of household responsibilities, maturity, life-satisfaction, use of time, future goals, and psychological well-being.

Major Findings: In general, one-parent/one-child families compared favorably with the other family forms studied. The women from these families were most likely to be working fulltime, most likely to be dating one person seriously, and most likely to be active in a range of educational and leisure pursuits. These women were also the most career oriented and the most planful about their future lives. The women who had adjusted least well to the divorce (perhaps because they were the least likely to have initiated the divorce) were those in the one-parent/two-child group. Divorced women with two and three children were more likely than women with one child to complain of feelings of depression, hopelessness, and anxiety. These women were also experiencing the most severe financial problems.

Only children from both the one-parent and two-parent families shared many characteristics that have been found to be associated with only children in other research. These children, compared to the children with siblings, were described as mature, independent, bright, and comfortable with adults. Children in one-parent/two-child families were more likely than other children to have a number of interpersonal, emotional, and behavioral problems.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and consequences of fertility.

Proposed Course: This research is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	The Personal Meaning of Voluntary and Involuntary Childlessness
Contract Number	:	NO-HD-82853
Contractor	:	American Institutes for Research in the Behavioral Sciences
Money Allocated	:	$47,981 (1978); $6,822 (1980)

Objectives: This study examines the antecedents to and personal meanings of childlessness within a marriage. In-depth psychiatric interviews will be conducted with individuals from 60 intact marriages. In one-half of the marriages the age of the wife shall be 35-40; in the other, the age shall be 50-55. Within each age group, one-half of the couples shall be childless by choice (NOC-V), and one-half childless as a consequence of an incapacity to conceive or bear children (NOC-I). A composite picture of the main pathways through which married individuals and couples arrive at a childfree state shall be drawn from analysis of in-depth interviews, and a picture presented of the personal meanings of this state near the end of the reproductive period and during the post-reproductive period shall be drawn. In addition, a descriptive account of the life course of childfree individuals and couples shall be developed. For comparison two additional groups of married couples shall be added: those that had adopted children (AdC) and those that had borne natural children (NatC).

Major Findings: A comparison of the voluntarily and involuntarily childless with respect to their developmental histories and the psychosocial factors affecting their current childbearing status shows a large "grey area" between the two extreme poles of voluntary and involuntary childlessness. In other words many couples are childless both because there are biological limitations on their childbearing and because of personal choice. Sometimes the biological and psychological factors act in concert at a single, critical decision point. In other cases they act in sequence so that the individual or couples is first limited biologically and later opts not to have children or vice versa.

Preliminary results indicate that the four groups, two childless and two with children, can be ordered with regard to their motivation for childbearing as follows: NoC-V < NoC-I < NatC and AdC. It appears that couples with adopted children are as a group even more motivated than some couples with natural children but additional analysis is necessary to confirm this observation.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants, and consequences of fertility.

Proposed Course: This study is expected to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	The Consequences of Being an Only Child: An Analysis of Project TALENT Data
Contract Number	:	NO1-HD-82854
Contractor	:	American Institutes for Research in the Behavioral Sciences
Money Allocated	:	$85,669 (1978)

Objectives: Data from Project TALENT (a longitudinal study of high school students, initiated in 1960 with follow-up data collection 11 years later) were used to investigate the consequences of being an only child. The investigators predicted, from a model based on previous research findings, that birth status would be related to subsequent development and achievement, but that the relation would be mediated by family background, environment and life experiences.

Major Findings: Only children and children with one sibling, all from intact, two-parent families, were compared. Few differences found were significant and none was large. "Onlies" were most like first-borns in two-child families and differed very little from them. However, an examination of trends showed that, relative to children from two-child families, only children: received higher scores on cognitive tests, were more mature, socially sensitive, and tidy; were more interested in white-collar, scientific, cerebral occupations and activities and less interested in blue-collar, manual, or outdoor occupations and activities; had a less intense social life; did not differ in health; spent more time in solitary, intellectual, and artistic activities; were more academically oriented in high school and showed greater academic achievement by age 29; married better educated spouses and had fewer children; worked less during high school and had higher long-term occupational and financial expectations; and did not differ in geographic and residential mobility.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility behavior. More specifically, the findings represent a needed contribution to objective information about only children and show little difference between "onlies" and first-borns.

Proposed Course: This project is completed.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Fertility Research Resource
Contract Number : 1-YO1-HD-91040
Contractor : National Technical Information Service
Money Allocated : $110,868 (1979)

Objectives: This project is for the generation of public use tapes with uniform documentation and their distribution through the National Technical Information Service (NTIS). The tapes involved are major United States surveys related to fertility, specifically: the National Surveys of Family Growth (NSFG), 1973 and 1976; the National Fertility Surveys (NFS) for 1965 and 1970; the Growth of American Families Surveys (GAF) for 1955 and 1960; and the June 1977 Current Population Survey (CPS) fertility and child care supplement. The Family Growth Surveys and the National Fertility Surveys will be structured into a hierarchical format in addition to the present rectangular format. Documentation will be standardized for the seven files and will include a machine readable CENTS-AID data base dictionary and a machine readable SPSS and OSIRIS codebook for the NSFG and NFS and GAF files. Comprehensive technical documentation also will be provided. An appendix will be created for the present CENTS-AID users' manual, illustrating procedures for using hierarchical files and will be based on NSFG and NFS examples. The NTIS also will provide a plan of distribution for the tapes once they are available.

Major Findings: All files, except the 1977 CPS, are being restructured into a hierarchical format. The manner in which the data were collected suggests three potential units of analysis: (1) respondents and their general attitudes about family size, contraception, etc.; (2) pregnancy intervals, including use of contraceptives and outcomes of pregnancies; and (3) live births. All files also are being restructured into a rectangular format at the pregnancy interval level. SPSS control cards are being prepared to serve as machine readable documentation for the rectangular files. A detailed users' directory is being written to describe the 1973 and 1976 NSFG data files, provide a thorough background of the two surveys, and index variables common to them. All files will have machine readable codebooks, prepared so that the CENTS-AID tabulation package can be used for analysis.

Significance to Biomedical Research and Program of the Institute: This project will provide an important new resource for persons wishing to study changes in fertility and fertility-related behavior that have occurred during the past 25 years.

Proposed Course: This project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Computer File of Studies on the Psychosocial Antecedents of Adolescent Pregnancy and Childbearing 1970-1980
Contract Number : NO1-HD-92800
Contractor : Western Washington University
Money Allocated : $122,509 (1979); $114,461 (1980)

Objectives: The purpose of this study is to construct a computer file of major studies of the psychological and social antecedents of adolescent pregnancy and childbirth. Nine studies conducted during the 1970s have been identified; however, others might be added. A major goal of the project is to produce an easily used data file available for secondary analyses. This will entail obtaining the data from principal investigators of the studies, establishing the quality of the data and the comparability of information from different studies, completing the file construction, testing the file, and writing a user's codebook and manual. Studies included in the file will be those that have measured psychological or social variables, have collected data on large samples of individuals aged 13-19, of either or both sexes, and have met accepted standards for scientific reporting of research methods and sampling.

Major Findings: Preliminary work has found a substantial degree of equivalence from one study to another in the measured variables. Up to 25 percent of the variables are equivalent across all studies, and even greater proportion are common to two or more studies.

Work continues on preparation of questionnaire and interview items for inclusion in the computer file, and on compilation of the user's manual. The manual will contain condensed reviews of salient findings for many substantive areas in the file, such as sexual knowledge and education, contraceptive use, and attitudes toward sex and contraception. The methods and problems of secondary analyses also will be summarized in the manual.

Significance to Biomedical Research and Program of the Institute: This project will make available to the scientific community a large data set containing information on an important biomedical and social topic--adolescent pregnancy and childbearing. Early childbearing has been shown to affect both the health and the social well-being of the mother and child through factors such as low birthweight, complications of pregnancy, mother's education, and socioeconomic status. The collection of data is both costly and time consuming, and frequently the data are analyzed only by the original investigator. Further analysis of previously collected data can aid in answering many questions in the area of adolescent pregnancy and childbearing.

Proposed Course: This project began in FY 1979 and is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	The Intellectual, Physical, Psychological and Social Consequences of Being Reared an Only Child
Contract Number	:	NO1-HD-92802
Contractor	:	Emory University
Money Allocated	:	$69,938 (1979); $71,756 (1980)

Objectives: This study undertook cross-sectional and longitudinal analyses of Cycles II (1964-65) and III (1966-70) of the National Health Examination Survey data on the only child compared with children in multiple-child families, to identify differences in characteristics between only children and others and to ascertain whether the differences were accounted for by mediating and control variables, such as parents' education and child-rearing practices.

Major Findings: This research found no negative effects on the physical, psychological, social, or cognitive development of an only child; nor did the research ascertain that "onlies" have any advantages over children in families with siblings. Using both cross-sectional and longitudinal data, the investigators analyzed the effects of other factors on the child's intelligence and achievement, including birth order, family size, income, mother's age at child's birth, parents' education, presence or absence of one or both parents, child's aspirations, parents' aspirations for the child, child's sex, race, and the interactions of certain variables. The investigators found no birth-order effects on intelligence and achievement test scores of children aged 6-11 when presence or absence of parents, parents' education, income, race, family size, mother's age at child's birth, and the interaction of presence of parents and parents' education were controlled. Differences in intelligence and achievement test scores of siblings aged 12-17 within the same families showed a slight advantage of first and second borns over later borns on the Wechsler verbal subtest for intelligence, but not for the block design test; the same was true for the reading achievement test, but not the arithmetic test. Rates of change in intelligence scores from ages 6-17 were constant, irrespective of race or social class. Parents' education and presence of both parents were the single most important factors in advantageous child development.

Significance to Biomedical Research and Program of the Institute: Considerable public and private interest centers on the main question addressed in this research: whether or not only children are different from or at a disadvantage to children with siblings. The research found no reasons why couples who wish to have just one child should not do so. The research makes an important contribution to the study of the consequences of fertility behavior.

Proposed Course: This project, initiated in FY 1979, is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Childlessness and Status Attainment
Contract No. : HO1-HD-92804
Contractor : University of Texas
Money Allocated: $40,974 (1979)

Objectives: The aims of this study divide into three groups: (1) the detailed demographic description of childlessness from 1910 to the present by cohort; (2) the development and implementation of a measurement strategy for separating the phenomenon of childlessness into voluntary and involuntary categories, and an attempt to see how these two categories have changed over time; (3) the assessment of the impact of voluntary childlessness on wives' status attainment variables such as family earnings and occupational prestige, and the comparison of the status attainment effects of voluntary vs. involuntary childlessness.

The investigator used a cognitive model, based on the woman's knowledge of her own fecundity, to separate the childless women into those who were voluntarily childless and those who were involuntarily childless. A non-pregnant woman with no children who knows that she is capable of having children yet plans to have none in the future is assumed to be voluntarily childless. On the other hand, a non-pregnant woman with no children who knows that she is unable to have children in the future is assumed to be involuntarily childless, as long as her inability to conceive does not stem from a sterilizing operation performed for contraceptive reasons. In this latter case, the woman is assumed to be voluntarily childless. A similar kind of model was developed for women with only one child. Using these models to interpret data from the 1973 National Survey of Family Growth, the investigator found that of the 903 childless white women, 71.5 percent were temporarily childless, 15.1 percent were voluntarily childless, and 13.4 percent were involuntarily childless. An analysis of the 1,009 white women with one child indicated that 54.5 percent were temporarily single childed, 32.1 percent were voluntarily single childed, and 13.4 percent were involuntarily single childed.

Significance to Biomedical Research Program of the Institute: This research requested under under RFP 78-9 relating to "childlessness and the one-child family," is important to the biomedical and behavioral programs of the Center and is policy relevant to departmental and Congressional concerns. It provides demographic descriptive data on childlessness and addresses the consequences of childlessness for individuals and families.

Proposed Course: This project is scheduled to get underway in FY 1979 and to be completed in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of the Consequences of Deciding to Remain Child-free, Compared with Deciding to Have Children and Deciding to Postpone Children.
Contract Number : NO1-HD-92805
Contractor : University of North Carolina
Money Allocated : $71,425 (1979)

Objectives: This project is analyzing consequences of remaining voluntarily childless. The voluntarily childless are being compared with postponers, undecided, and parents to test the hypothesis that voluntarily childless wives will be more likely to: (a) emphasize the costs of children, negative motivations for parents having children and positive motives for remaining childless; (b) be characterized by dyadic withdrawal; (c) have an egalitarian division of labor in the home; (d) have consistently high achievement for the wives; (e) have high marital satisfaction; (f) have higher combined income, less debt, and higher satisfaction with their standard of living. The study is also investigating whether voluntary childlessness is a qualitatively unique phenomenon or a quantitative extension of a model that explains low parity processes. Analyses are being performed on extant data from a random sample of 186 voluntarily childless and 598 other married women.

Major Findings: Preliminary analyses have found that the three childless groups have significantly higher marital satisfaction than the parents. The predictors of marital satisfaction, which included variables related to satisfaction with husband's economic provision, the woman's education, and dimensions of the couples' division of labor, were similar for the mothers and the postponers. Most differences between the postponers and the parents were due to differences on individual variables in the model. The processes leading to marital satisfaction for the voluntarily childless were different from those of the parents and the postponers. Both spouse involvement variables and satisfaction with husband's economic provison significantly influence marital satisfaction among the undecideds. The women in the three childless groups did a significantly smaller proportion of the total housework than the mothers. However, a substantial similarity was found between the sets of variables predicting division of labor for the parents and the postponers. The predictors of division of labor among the voluntarily childless were essentially the same as those for the undecideds.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants and consequences of fertility.

Proposed Course: This study will expire in FY 80.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Differential Consequences in Early Adult Life of Having Been an Only Versus a Sibling Child
Contract Number	:	NO1-HD-92806
Contractor	:	Bowling Green State University
Money Allocated	:	$68,803 (1979)

Objectives: Extant data from the 1973 National Center for Health Statistics, National Survey of Family Growth, were used to investigate the consequences in later life of having been an only child, for a national probability sample of women aged 15-44. Specific topics addressed were the differential consequences of "only" versus "sibling" children on educational attainment, family formation, and labor force participation.

Major Findings: The research results showed significantly higher levels of educational attainment among whites who were only children, compared with other whites, but no significant differences in this respect among non-whites. Adults who were only children and adults with siblings showed virtually no differences in family life (age at marriage, incidence of premarital pregnancies, length of birth intervals, number of pregnancies and children ever born, unwanted pregnancies and children, and desired and expected family sizes). The research also found very little support for the idea that adults who were only children are more likely than other adults to experience a divorce. Comparison of the two groups on economic variables showed no significant differences in duration of employment, occupational prestige, or levels of personal and family income. With respect to health indicators (fetal losses, anemia, high blood pressure, and complications of pregnancy), the differences between the two comparison groups were negligible. The researchers concluded that a decision to have more than one child so as to avoid having an only child may be misguided, since "onlies" differ vary little from children with siblings.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility behavior. More specifically, the findings represent a needed contribution to objective information about only children.

Proposed Course: This project is completed.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Long Term Consequences for Couples of Childlessness and One-Child Families
Contract Number	:	NO1-HD-92807
Contractor	:	The Pennsylvania State University, University Park
Money Allocated	:	$59,812 (1979); $31,562 (1980)

Objectives: This research utilized a 24-year longitudinal study of married women in Pennsylvania to evaluate the consequences for parents of being childless or of having only one child and also to examine several antecedent factors. Some 915 women from rural backgrounds, married only once and still living with their husbands, were interviewed as high school sophomores in 1946 (at about age 16), in 1957, and again in 1971. The data set, which included 45 childless women and 75 currently married women with one child, permitted an examination of the consequences for parents of childlessness and the one-child family. Antecedent factors, such as personality, family of orientation and birth order, and occupational aspirations, were available from the early data set. Consequences, including occupation and income aspirations and achievement, life satisfaction, aspiration for social mobility, and social participation, were available in the last data set.

Major Findings: The initial analysis addressed this question: are women who remain childless or have an only child identifiable on a series of adolescent personality traits and background characteristics? The answer was a qualified "no". Childless women in the sample were with a single exception, involuntarily childless. Thus the lack of selectivity among these women could be generalized only to the involuntarily childless. A clear finding from the analysis was that women with an only child were more similar to higher parity women than to the involuntarily childless women in personality traits and background characteristics.

Preliminary analysis of consequences showed a similar pattern: The differences were primarily between childless women and all other women. As an example, social mobility aspirations were very similar for women with one or more children, and similar for women who had borne fewer or more children than were planned early in marriage. Childless women, however, had significantly higher mobility aspirations. Since these women had about the same occupational aspirations as other women at age 16, their higher mobility aspirations as adults appeared to result from being childless. Additional work is under way to determine if this finding will hold when other current characteristics and background variables are controlled.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the biomedical and behavioral programs of the Institute in its focus on the consequences and correlates of fertility behavior.

Proposed Course: This project is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sexual and Contraceptive Attitudes in Behavior of Young, Single Females
Contract Number : NO1-HD-92809
Contractor : The University of Guelph
Money Allocated : $26, 354 (1979); $14,119 (1980)

Objectives: This project involved analysis of two data sets on adolescent sexual activity and contraception. Both data sets were collected in Ontario, Canada; one was from a film evaluation study of 447 females ages 17-22, and the other from a birth control clinic study of 487 females aged 13-20. The women were classified three ways: as virgins unlikely to have premarital intercourse; as virgins likely to have premarital intercourse; and as non-virgins. The research focused on measurement and prediction of contraceptive use, and embarrassment about obtaining contraceptives.

Major Findings: The variables significantly related to virginity status were age, religiosity, career aspirations, parents' acceptance of premarital intercourse, peers' acceptance of premarital intercourse, peers' experience with premarital intercourse, commitment to dating, and frequence of dating. The results of discriminant analysis showed that peers' experience with premarital intercourse was the most important predictor of virginity status, followed by commitment to dating and religiosity. Contraceptive use was measured in seven ways. The results of multiple regression analysis for each measurement showed the most significant predictor of contraceptive use to be belief in the necessity of practicing birth control. However, more variance was explained in the film evaluation sample than in the clinic sample. The results suggest that the measurement of contraceptive use and the type of sample studied can influence research findings. A scale of items was constructed to measure embarrassment about obtaining contraceptives. Embarrassment was greater near the parental home than distant from it. Least embarrassment was found for obtaining birth control pills and most for condoms. Guilt about sexual activity and ease of access to contraceptives were among the most important influences on embarrassment or the absence of it. Parents' attitudes toward premarital intercourse had the most important effect on embarrassment about obtaining contraceptives near the parental home, but away from home friends' attitudes were more important. Finally, embarrassment was significantly related to the type of contraceptive used at last intercourse and to whether or not contraceptives were consistently used.

Significance to Biomedical Research and Program of the Institute: Adolescent pregnancy is a research area of high priority for the Institute. This research on sexual and contraceptive behavior pertains directly to the antecedents and prevention of adolescent pregnancy.

Proposed Course: This project was funded in FY 1979 and is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on Childlessness and the One-Child Family
Contract Number : NO1-HD-92814
Contractor : Steiger, Fink, and Kosecoff, Inc.
Money Allocated : $39,719 (1979); $50,674 (1980)

Objectives: This research will assess the social-economic, psychological, and physical effects of parity (number of children born) on couples who either have completed their childbearing or are in the later years of childbearing. The social-economic variables to be examined are social adjustment, friends and social networks, career patterns, and income. The psychological variables are social and psychological well-being, depression, and perceived quality of life. The physical variables are current health status, resistance-susceptibility to illness, and concern about health. Couples will be divided into three groups, based on the wife's age (30-39, 40-49, and 50-59), so as to obtain information about changes in the effects of parity during the life cycle. The data to be used are from the RAND Corporation's Health Insurance Study (HIS), which included more than 8,000 individuals (2,750 families) in four geographic areas of the United States. Some additional data needed to ascertain the voluntary or involuntary nature of fertility will be collected by RAND. Analytical techniques will include discriminant function analysis and multiple regression.

Major Findings: The major activity during the first year of the project was the development of four data files containing information from RAND's HIS. Other activities included construction of indices summarizing the more than 500 variables available for each individual, and identification of the sample to be analyzed. Twenty percent of the couples in the sample are childless; 10 percent have one child; 45 percent have two or three children; and 25 percent have four or more children.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interests in the consequences of fertility behavior. Childless couples and those with only children are still comparatively rare in the United States, and they tend to be negatively stereotyped as selfish, maladjusted, lonely, unhappy with marriage, etc. This research will use an excellent data source to provide objective information about childless and one-child families, including previously unavailable information about physical health.

Proposed Course: This project began in FY 1979 and is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Disruptive Effects of Fertility: A Longitudinal Study of the Sequential Consequences of Childlessness and Childbearing on the Educational and Occupational Pursuits of College-Educated Women
Contract No. : NO1-HD-92816
Contractor : DePaul University
Money Allocated: $42,098 (1979)

Objectives: This research studied the effects of being an only child, of having one or more children, and of being childless on such variables as educational and occupational aspirations and attainment. The goal was to develop a parsimonious model of initial aspirations, fertility and occupational choices, and the relationships between these. Data were from the National Opinion Research Center's longitudinal survey of 1961 college graduates. The first wave was conducted in 1961 on a representative sample of 41,116 graduates; a subsample of 4,868 graduates completed follow-up waves in 1962, 1963, 1964, and 1968. The present research used data from a subsample of 1,685 female graduates.

Major Findings: Having children was generally negatively related to continuity of employment, as well as to occupational aspirations and attainment. Childless women were more likely to have uninterrupted employment (1962-1968) than were mothers. Also, the first child's birth was negatively related to full-time labor-force participation and to occupational aspirations four years later, even without additional births. Women with one child were more likely to be employed in 1968 than were women with two or more children; however, women in the former group had lower occupational attainment and were less likely to have exceeded their initial aspirations. This finding was attributed to the possibility that the former group intended to have more children, whereas the latter group did not.

The occupational attainment of childless single women did not differ systematically from that of childless married women, although in 1968 the former group had somewhat more graduate education, higher occupational aspirations, and higher occupational attainment relative to initial aspirations.

Women reared as only children differed little from other women in fertility and occupational outcomes, but the women who were "onlies" tended to have greater continuity of employment, higher occupational aspirations, and fewer children by 1968.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility behavior.

Proposed Course: This project is scheduled for completion in FY 1980.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Reciprocal Interaction Skills and Ego Identity Formation in Adolescents
Contract Number: NO1-HD-92819
Contractor : The University of Texas at Austin
Money Allocated: $79,380 (1979); $72,122 (1980)

Objectives: The study proposes to analyze data on 40 only children (half of each sex), 80 first children, and 80 second children from two-child families. Half of the second and third children will have siblings of the same sex, and half will have siblings of the opposite sex. Three clusters of variables believed to mediate the consequences of being an only child will be examined. These are: parental attitudes concerning family size and achievement; sibling and peer interaction; and family interaction and climate. Because only children may have different experiences than children with siblings relative to these three factors, it is possible that they will exhibit distinctive patterns of ego identity and reciprocal interaction skills. The investigators will also study the relationship of process and status to each other and their joint effects.

Major Findings: Preliminary findings show that progress has been made in conceptualizing family interaction. The researchers have identified a quality of family interaction: self-assertion of one's own views; validation or respect for others; and permeability or responsiveness to others. In an individuated family, each member manifests these qualities rather than showing specialization by one member. As adolescents' feelings are asserted and acknowledged, the sense of self is clarified and role-taking and communication skills useful with peers are strengthened. Procedures are now established by the investigators for analyzing interaction by sequentially coding messages and scoring distinctive properties of individuals, dyads, and the whole family.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the consequences of fertility.

Proposed Course: This study is expected to continue into FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : "Sex Role Development and the Single Child Family"
Proposal Number: NO1-HD-92820
Contractor : Institute for Research on Social Problems
Money Allocated: $93,285 (1979); $96,169 (1980)

Objectives: The major objective of the proposed research is to study sex-role development, together with its parental antecedents, in only children. Attention will be given to the consequences of being an only child for sex-role development and identity. Also, the effects of having an only child on the parents' family planning decisions and attitudes toward sex roles will be considered. The research will explore the strength of the parents' need for child gender diversity, the relation of this to family size decisions, and the parents' sex-role expectations in relation to family planning decisions.

The investigator will first compare the parents of only children with the parents of two children in such characteristics as sex-role attitudes, degree of sex-typing, perceptions of their child's sex-role orientation, and factors related to family size decisions. Secondly, the study will compare the sex-role identity of the only children with both the first-and last-born children at the pre-school (ages 4-6) and fourth grade (ages 8-10) levels.

Major Findings: Twenty-four different elementary schools, nursery schools, and day-care centers have participated in the study. Data collection at two grade levels is almost complete. A total of 295 children have been tested: 149 at the kindergarten-preschool level (ages 4-6), and 146 at the third and fourth grade level (ages 8-10). An additional 20 grade school children have been tested on the behavioral and retest measures, which will lend behavioral validation and psychometric support to the measures.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the social, psychological, and economic consequences of childlessness or of having one child.

Proposed Course: This research began in FY 1979 and will continue into FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Study of Consequences of Remaining Childless or Having Only One Child
Contract Number: NO1-HD-92821
Contractor : Research Triangle Institute, Research Triangle Park, N. C.
Money Allocated: $199,074 (1979); $87,006 (1980)

Objectives: The investigators will (1) analyze the 1973 National Survey of Family Growth and the 1971 and 1975 Current Population Surveys and (2) hold discussions with 60 couples to assess the long-term social, economic, and psychological consequences of childlessness and of having only one child rather than two or more. The research will examine the adult life experience, focusing on important economic, demographic, and social-psychological aspects of the quality of life: (1) marital dissolution and remarriage; (2) female labor-force participation and occupational attainment; (3) family income and standard of living; and (4) the degree of congruence between women's educational and occupational attainments, and between spouses' occupational attainments. The researchers will also examine in depth the ways in which couples experience the "costs" and "benefits" of remaining childless or of having one child or several children. The analyses of large-scale studies of the "objective" aspects of quality of life will be combined with in-depth studies of couples' experiences in these areas and in marital and parental roles, friendships, and use of financial resources and leisure time.

Major Findings: Preliminary findings suggest the importance of childlessness and parenthood for men's as well as women's careers: among black couples, voluntary childlessness appears to be positively associated with male occupational attainment, whereas involuntarily childless white males were found to have lower than expected occupational attainment, possibly because of poor health. Childlessness does not appear to increase the probability of divorce, for either blacks or whites; but for whites the presence of preschool-age children appears to lower the probability of divorce during the next five years. For white women aged 55 or older in 1975, childlessness does appear to have been associated with a higher probability of divorce from a first marriage at ages 30-35.

Significance to Biomedical Research and Program of the Institute: This research is important to the biomedical and behavioral programs of the Institute and is policy relevant to departmental and Congressional concerns.

Proposed Course: This project is scheduled to be completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Societal Consequences of Adolescent Childbearing
Contract Number : NO1-HD-92822
Contractor : The Urban Institute
Money Allocated : $117,398 (1979); $199,579 (1980)

Objectives: The proposed research will provide information at the national level on public expenditures to teenage parents through various programs: AFDC, Medicaid, Food Stamps, etc. Three types of estimates will be calculated. First, there will be calculation of actual expenditures for a given year (1975) on births to teenagers and non-teenagers. Next, the 1975 data will be projected forward to 1990 using a number of different assumptions (7 in all). These assumptions relate to different probabilities of birth among teenagers and others, different assumptions about illegitimate pregnancy, and school dropout rates among teenagers if they get pregnant. The third estimate will cumulate over the period 1975 to 1990 the cost to the government per mother for teenage versus non-teenage mothers who bore their first child in 1975. This group of teenage mothers will be treated as a cohort and the cost of their cumulative fertility to the government will be examined.

Significance to Biomedical Research and Program of the Institute: The project offers an ideal opportunity to assess the public sector costs of adolescent childbearing, a topic of considerable interest to the Institute with both social and biomedical implications.

Proposed Course: This project is scheduled for completion in FY 1982.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Estimating and Forecasting Money Expenditures on Children
Contract Number : NO1-HD-92823
Contractor : MATHTECH, Inc.
Money Allocated : $94,802 (1979)

Objectives: This project will analyze the cost of children based on the economic theory of indirect utility as embodied in a transcendental log functional form. The investigators are using the 1972-1973 consumer expenditure survey as the basis for estimating the cost of children, and combining these cost estimates with a separate analysis of life cycle income to predict cost of children from the year 1980 through 2000.

Building on consumer demand theory, demand functions are to be estimated in which consumption is a function of prices and income. The resulting demand functions are substituted back into the utility function, producing an indirect utility function. The transcendental logarithmic indirect utility function is adopted as the functional form. This combined with Roy's identity permits derivation of the implied consumer demand functions for each commodity. Lifetime income profiles for head and spouse are being developed and used in the total expenditures equation to forecast expenditure category. The marginal cost of an additional child is then to be computed.

Three sets of procedures are used to estimate the marginal maintenance cost of a child born in 1980 until the child achieves financial independence: Espenshade's methodology with several standard of living measures; Turchi's methodology with more narrow expenditure categories; and a methodology based on a relative expenditure systems approach. Results from the three methodologies are to be compared.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility.

Proposed Course: This project is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Estimating the Cost of Children in the United States
Contract Number : NO1-HD-92824
Contractor : Carolina Population Center, University of North Carolina
Money Allocated : $119,553 (1979); $81,213 (1980)

Objectives: Three different methodological approaches will be used to estimate the direct, out-of-pocket costs of rearing children to an age of financial independence. Each method will utilize the 1972-1973 Consumer Expenditure Survey data for preparing basic estimates, supplemented in some instances with other data, such as the 1976 Health Interview Survey. The first method will estimate child costs in terms of additional family income required to maintain a given standard of living as children are born and mature ("a standard of living" approach). The second method constructs unit consumer scales and estimates child costs in terms of unit proportions for each category of expenditure (an extension of the "unit consumer scale" approach). The third method makes use of recent developments in demand system analysis that incorporate demographic variables directly into household demand equations (a "utility-based" approach). A major aspect of the proposal will be comparative assessment of the strengths and weaknesses of the different approaches. In addition, 1972-1973 data will be compared to 1962 data for examination of trends and change. Each method can be used to provide cost data by year of child's age and by a variety of family characteristics, including family size, family income, age of head, race, occupation and education of family head, region and type of residential area. Projections of costs of rearing a child born in 1980 to age 22 will be calculated using three different assumptions of future inflation trends. Estimates will be disaggregated by age of child, family size, age of parents, family income, race, occupation, education, region, city-size, and life cycle changes in family income.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility.

Proposed Course: This project is scheduled for completion in FY 1982.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Research on the Economic Determinants of Fertility
Contract Number	:	NO1-HD-92825
Contractor	:	Data Resources, Inc.
Money Allocated	:	$84,371 (1979); $60,051 (1980)

Objectives: Cross-sectional estimates of the costs of maintaining a child through its dependent years will be calculated using data derived from the 1972-1973 Consumer Expenditure Survey Summary and Detailed Interview tapes. Estimates of child costs will be made by selected family characteristics including family size, family income, age of parents, residential area, occupation and education of family head. Separate estimates of expenditures will be made by broad categories of consumer goods and services such as food, housing, medical care, and education. The allocation of expenditures by category among family members will be made using a modification of a method developed by Edward Lazear of the University of Chicago and Robert Michael. The cross-sectional estimates will be used as the basis for forecasting year-by-year expenditures for a child born in 1980 through its period of dependency. The forecast method will utilize an age-income model developed by Data Resources, Inc. which is linked to the agency's macroeconomic model of the U.S. economy. Separate forecasts will be developed for families of different characteristics, including three levels of income. Forecasts of summated costs will be expressed in discounted and undiscounted form under three different assumptions of inflation trends.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility.

Proposed Course: This project is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Societal Consequences of Adolescent Childbearing
Contract Number: NO1-HD-92826
Contractor : Analogs
Money Allocated: $109,705 (1979); $54,771 (1980)

Objectives: The research will result in the development of computer simulation methodology for estimating public sector costs consequent to adolescent childbearing as a function of current or projected birth rates by age of mother, various rules for determining which costs should be associated with adolescent childbearing, and various life cycle periods. This methodology will be used to estimate public sector costs and the model will assess the sensitivity of these costs to various rules for making associations of cost factors with events following adolescent births.

In addition to total costs associated with a current year's birth cohort the estimation methodology will be able to accept projected fertility rates and will also be able to develop confidence limits on estimates as well as ranges for various life cycle periods and assumptions regarding "associated costs" such as those consequent subsequent births to past teenage mothers now on welfare. The methodology will be validated by comparing predictions of future costs based on past birth cohorts with current AFDC caseloads and associated public sector costs.

Significance to Biomedical Research and Program of the Institute: The project offers an ideal opportunity to assess the public sector costs of adolescent childbearing, a topic of considerable interest to the Institute with both social and biomedical implications.

Proposed Course: This project is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Being and Having an Only Child on Intelligence, Interpersonal Orientation, Attitudes and Time Use
Contract Number: NO1-HD-92830
Contractor : The University of Texas at Austin
Money Allocated: $134,575 (1979); $76,941 (1980)

Objectives: This research comprises a series of three interrelated studies to assess the consequences of being and having an only child on intelligence, interpersonal orientation, attitudes, and time use, over a two year period. The research is designed to examine the consequences of growing up without siblings particularly (but not solely) on the development of interpersonal skills and IQ. The research studies are grounded on the confluence model of intellectual development proposed by Zajonc and Markus, which accounts for the relationship between family size and intelligence by explaining the IQ discontinuity of only children as caused by the fact that only children do not have younger siblings to tutor.

Study 1, involving 144 families (48 one-child families and 96 two-child families), will examine and compare the activities of family members, and the intellectual abilities and personalities of the children. The study is expected to provide descriptive data about one- and two-child families. Study 2 is designed primarily to examine the effects of teaching someone younger on intellectual abilities of the teacher. Older subjects, 15- and 16-year-olds, will teach younger subjects, 14- and 15-year-olds. Only children will be compared with first and last borns from two-child families. Because the predicted differences do not reliably appear until adolescence, the subjects for these studies will be that age group. Study 3 will involve 360 female undergraduates and is primarily designed to assess the cumulative effects of repeatedly teaching someone younger on intellectual abilities.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interest in the antecedents, determinants, and consequences of fertility.

Proposed Course: This study will continue through FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Contraceptive Decision Making in Adolescent Couples
Contract Number : NO1-HD-92835
Contractor : American Institutes for Research in the Behavioral Sciences
Money Allocated : $54,510 (1979); $43,228 (1980)

Objectives: This study will investigate couple communication and decision making regarding contraception among young unmarried sexual partners. It is planned to examine factors which influence these interactions in teenagers and to explore the nature of the effects of such communication on fertility decision making and actual contraceptive practices. Among the questions which the research will address are the following: (1) Why do some couples communicate while others do not? (2) What are the dynamics of the interaction process? (3) Does communication facilitate decision making about contraceptive behavior and in what way? (4) What role do demographic, situational, interpersonal and psychological factors play in communicating, decision making and contracepting behaviors? The goal is to provide a sufficient level of understanding of the processes underlying communication and decision making in teenage couples, as they relate to contraception, to permit the development of a theory and model for these phenomena.

The data for the study will be collected by means of in-depth interviews with 80 male and female sexual partners (160 total) in the greater Boston area. Two subsamples of 40 couples each, based on the age of the young women, will be selected: those aged 15 or 16 and those aged 17 or 18. Included in the data gathering will be demographic factors, situational factors including the quality and depth of information on contraceptives, acceptability of various contraceptives, timing of communication between the couple, institutional membership including group and club membership, informal groups, the impact of peer groups, interpersonal relationships with a focus on the degree of couple intimacy, the degree of agreement about who should control the contraceptive, congruence of attitudes and concern about pregnancy, back-up in case of contraceptive failure, and psychological variables including perception of the ability to control fertility, the extent to which the couple sees a risk of getting pregnant, etc. Heavy reliance will be placed on instrumentation developed by Miller, Furstenberg, Cvetkovich, and others. The interview schedule will consist of a combination of structured and open-ended questions. Data analysis will be both quantitative and qualitative.

Significance to Biomedical Research and Program of the Institute: This project pertains directly to the Institute's interest in contraceptive behavior.

Proposed Course: This project is scheduled for completion in FY 1981.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Marriage and Fertility Histories, July 1980
Contract Number : YO1-HD-01044
Contractor : Bureau of the Census
Money Allocated : $275,000 (1980)

Objectives: This project is for the collection of data on marital history and living arrangements and support of children in the June 1980 CPS supplement. The June CPS includes fertility history and expectation data in addition to the usual socio-demographic data such as education, occupation, income, race, history, etc. In many respects this will replicate undertakings in 1971 and 1975 where marriage and fertility history data were collected. Marriage history data has been collected from all subjects 15-70, fertility histories from women 15-70 and fertility expectations from women 18-39. Men who have been married more than once were asked if they have children from a previous marriage(s) living elsewhere, how many, and whether the father provided financial support for such children. Following data collection, the Census Bureau will provide NICHD with a public use data tape.

Significance to Biomedical Research and Program of the Institute: With marital disruption rates at an all-time high and fertility at a similar low, research on the timing, correlates and interrelationship of marriage and fertility behavior is of very high priority and directly relevant to the Institute's interests in the determinants and consequences of fertility.

Proposed Course: This project will be completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Contraceptive Decisions: Spousal Relationship, Method Commitment, and Expectancy-Values
Contract Number	:	NO1-HD-02802
Contractor	:	Center for Consumer Research, University of Florida
Money Allocated	:	$72,639 (1980)

Objectives: This research will integrate three areas of investigation related to contraceptive choices and practice: (1) theoretical and empirical efforts in the area of expectancy-value formulation; (2) joint consumer decision making by marital partners; and (3) theory and research on the nature of the marital relationship (decision making style). The purpose is to analyze the impact of joint decision making on contraceptive use (continuous, episodic, or non-use), for different contraceptive methods. Three interviews within 12 months will be conducted with 360 sexually active white couples who are not planning a pregnancy. In half the couples the women will be aged 18-27; in the other half they will be aged 36-45. Both partners of each couple will be interviewed individually and jointly, to ascertain each one's expectations and values, perceptions about the other partner's expectations and values, and joint decision making. The analysis will focus on the explanatory power of the expectancy-value conceptual framework, combined with joint decision making, partners' perceptions, influence of marital decision making style, and type of contraceptive commitment as these influence contraceptive choices and ultimate fertility intentions.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the antecedents and determinants of fertility.

Proposed Course: The research began in FY 1980 and will continue into FY 1982.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Interdisciplinary Assessment of Fertility Management in a High Fertility Community
Contract Number : NO1-HD-02803
Contractor : Department of Community Medicine, University of Kentucky
Money Allocated : $129,301 (1980)

Objectives: This interdisciplinary three-year project aims at describing fertility regulating practices, attitudes and perceptions, as well as the factors associated with these variables, in a high fertility Eastern Kentucky Appalachian county of about 13,000 people. Three strategies will be employed. First, a "community inventory" will be undertaken to determine general access to fertility regulation information, supplies, and services, and to assess the views of community leaders and the general community atmosphere regarding family planning; this will involve structured and unstructured interviews and a traditional ethnographic approach. The second strategy involves survey interviews with 400 married females, and 100 of their husbands, covering knowledge and attitudes regarding family planning, fertility history, family conjugal roles, value and personality factors, and socio-demographic variables. The third strategy entails in-depth interviews, based on factors which emerged using the other two strategies, with 100 women and their husbands. Analyses of the data will be both descriptive and exploratory.

Significance to Biomedical Research and Program of the Institute: This research should provide insights into the dynamics of fertility regulation that are unobtainable by single disciplinary approaches. The results are likely to increase understanding of the complex of variables that affect birth planning at the community, family, and individual levels.

Proposed Course: This project is scheduled for completion in FY 1983.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Consequences of Childlessness for Older Men
Contract Number: NO1-HD-02804
Contractor : The University of California at Los Angeles
Money Allocated: $56,387 (1980)

Objectives: This is a study of the psychological, social, and emotional consequences of childlessness among married men 60 years of age and over. It is hypothesized that the relation between parity (childless or parenthood) and well being is moderated by social-psychological variables such as social contact and congruence between fertility desires and outcomes. One hundred-fifty men over age 60 with children will be compared with 150 childless older men on a variety of variables including several measures of well-being, congruence between desired and actual family size, satisfaction with family size, expectations of parity outcomes and actual outcomes, social interaction, and socio-demographic variables.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interest in the consequences of fertility.

Proposed Course: This study is expected to continue into FY 1982.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on the Societal Consequences of Adolescent Childbearing Public Assistance Costs, 1975 and 1977
Contract Number : NO1-HD-02837
Contractor : JWK International Organization
Money Allocated : $90,845 (1979)

Objectives: This project uses the 1975 and 1977 AFDC Recipient Characteristics Surveys for an analysis of the costs of teenage childbearing among the population receiving public assistance. The direct dollar costs of being a prior teenage mother are being obtained for AFDC recipeints aged 20 and 30 for AFDC payments, food stamps, other state and local assistance payments, and emergency payments. The indirect impact of teenage mother motherhood on these same costs will also be explored. Causal models including intervening variables such as number of children, educational level, occupation, and marital status will be used to compute the incremental costs associated with each intervening variable. Ultimately, the dollar amount of each indirect effect will be calculated as a proportion of those costs directly related to each intervening variable and the costs due to teenage childbearing -- direct plus indirect -- will be summed. For AFDC mothers currently in their teens, total direct costs will also be cumulated and indirect costs will be explored. In addition, reliance upon Social Services will also be tabulated. Finally, development of a causal model is proposed to examine the number of months mothers have received AFDC payments.

Significance to Biomedical Research and Program of the Institute: This project addresses an important consequence of adolescent fertility. It has high policy relevance for HHS programs.

Proposed Course: This 18-month project began in FY 80.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Center for Population Research
Reproductive Sciences Branch

Within the context of the mission of the Center for Population Research (CPR), NICHD, the Reproductive Sciences Branch (RSB) facilitates the acquisition and application of the knowledge required to enable men and women to: 1) control their fertility; and 2) free themselves from reproductive disease and disablement. The primary means by which such facilitation of the CPR mission is accomplished by the RSB is through the award of grants for the support of research and training in the reproductive sciences. During the period from October 1, 1979 to September 30, 1980, the RSB budget of approximately $41,090,000 was used to provide grants and other forms of support for activities subsumed by the following programs: 1) Fundamental Biomedical Research on Problems of Human and Relevant Animal Reproduction; 2) Institutional Programs in Multidisciplinary Reproductive Sciences Research; 3) Reproductive Sciences Research Manpower Development; and 4) Reproductive Sciences Research Facilitation and Augmentation.

1. Fundamental Biomedical Research on Problems of Human and Relevant Animal Reproduction

"Internalization" of Gonadotropins. The Reproductive Sciences Branch attaches great significance to research aimed at the elucidation of mechanisms of action of reproductive hormones. It seems almost axiomatic that knowledge of the mechanisms by which hormones exert their effects on the body will serve as the basis for the development of fully satisfactory methods of fertility regulation as well as for the alleviation or cure of infertility. Because advances in research on the mechanisms of action of reproductive peptide hormones have lagged behind those for steroid hormones, we are quite pleased by a recent "breakthrough" in research on gonadotropins. The gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), control ovulation and hormone secretion by the ovary as well as sperm production and hormone secretion by the testis. It has long been a tenet of reproductive endocrinology that gonadotropins bind to target cell surfaces and do not gain admittance to the interior of such cells. In recent months, RSB grantees have provided abundant evidence of the "internalization" of gonadotropins. It is not known at this time whether the process of gonadotropin internalization leads only to degradation of the hormone or whether it has an obligatory role in the mechanisms by which the hormones exert their regulatory actions on cells. Regardless of whether the end result is regulation or degradation, the recently revealed process of gonadotropin internalization by target cells points toward an entirely new field of investigation which may lead to acceleration of research progress in the elucidation of the mechanism of action of gonadotropins.

Gonadotropin Releasing Hormone (GnRH) Receptor. The results obtained during the past year have shown that it is now possible to accurately and reliably demonstrate GnRH receptors in the pituitary gland and hence, to delineate the role of membrane GnRH receptors in determining the subsequent gonadotropin

response to the hypothalamic hormone. This has provided us with the first available system to accurately measure GnRH receptor affinity and number. Therapy with GnRH, or the superactive GnRH analogs, causes a reduction in both pituitary gonadotropin and gonadal steroid secretion. This may be due to "down regulation" of pituitary membrane receptors. Analysis of GnRH receptors will allow us to answer this specific question. Moreover, the exciting discovery of specific GnRH receptors in the ovary and the testes allows further studies to elucidate whether GnRH or analog therapy has a direct effect on ovarian and testicular receptors and steroidogenesis. It has been documented that GnRH therapy results in decreased steroidogenesis by the gonad, but until the demonstration of specific GnRH receptors, the mechanisms by which this was achieved was unknown. It will now be possible to look at changes in GnRH gonadal receptors and determine whether this correlates with changes in steroid hormone synthesis. Long acting GnRH analogs are being developed as possible contraceptives and represent an exciting advance in the development of new contraceptive measures.

New Action of FSH. A new model has been developed for studying the mechanism of action of FSH, i.e., the ability of FSH to stimulate incorporation of labeled glucosamine into mucopolysaccharides of proteoglycans secreted by ovarian granulosa cells. The stimulation of this biosynthesis appears to be specific for FSH and, therefore, offers an attractive possibility of being developed as a new bioassay for FSH, a methodology that would be very useful for understanding the relative roles of the different gonadotropins in reproductive physiology. Work from other laboratories investigating the effects of FSH on cultured Sertoli cells of the testes shows that Sertoli cells have the capacity to synthesize proteoglycans, thus sharing this property with follicular granulosa cells.

Prostaglandins (PGS) and Ovulation. Studies are progressing on the elucidation of the biochemical mechanisms involved in the process of ovulation, specifically on the role that prostaglandins play and the mechanism by which the follicular wall ruptures. It had been shown previously that PGS increase markedly in rabbit Graafian follicles as ovulation approaches. The follicular fluid contains 4-10 times more PGS than the granulosa or theca cells or the oocyte. These rabbit granulosa cells had 4 times the capacity of the theca wall to synthesize PGS. Use of a model system to study the control of PG synthesis in isolated granulosa cells showed that LH caused a 5-fold stimulation of prostaglandin synthetase activity. This finding of an *in vitro* effect of LH on isolated granulosa cells of rat follicles was confirmed in two *in vivo* systems. GnRH analogs have been shown to have a direct stimulatory effect on ovarian PG accumulation *in vivo* and *in vitro*. Prompted by these findings, the investigators examined the effect of GnRH and certain agonists on oocyte maturation. It appears that oocyte maturation can be induced in follicle enclosed oocytes (isolated from PMSG treated immature rats) incubated with GnRH or certain analogs. This interesting preliminary observation of the direct effect of GnRH on the oocyte awaits further documentation.

Implantation. Although histamine has long been considered as an important factor for the induction of implantation or decidualization, the evidence for this has been rather weak. Recent studies in rabbits reveal that blastocyst cell membranes carry histamine receptors and that blastocysts have the

capability to produce histamine because they contain histidine decarboxylase. This enzyme is not found in the endometrium. Therefore, it may be that histamine is produced by the blastocyst and stimulates the endometrium locally for implantation.

Fertilization. At fertilization the spermatozoon binds to the ovum. It has been thought that the binding of the spermatozoon to the ovum involved membrane-bonded acrosome granules. Now it has been shown that these granules contain a sperm-specific protein named bindin. This protein has, for the first time, been isolated, purified and its molecular weight determined to be 30,500. Its amino acid sequence is currently being investigated. At fertilization ova release a proteolytic enzyme from their cortical granules. The breakdown of bindin by the released enzyme may be one of the mechanisms by which polyspermy is blocked.

GnRH Analogs in Female Fertility and Infertility. Perhaps the most significant new developments in terms of potential practical application are those associated with the synthesis and use of analogs of GnRH. GnRH, also known as Luteinizing Hormone Releasing Hormone (LHRH), is the acronym for Gonadotropin Releasing Hormone. GnRH is normally produced in the brain and stimulates the pituitary gland to secrete gonadotropin(s) which, in turn, induce ovulation. Thus, some GnRH analogs are included in the category of "fertility" drugs about which so much has been written. Some GnRH analogs, known as "super" agonists, are 1000 times more powerful than natural GnRH in their capacity to elicit the secretion of gonadotropin(s) by cells of the pituitary gland. Normally cycling women who received injections of 50 micrograms of a long-acting GnRH analog on each of two successive days during mid-luteal phase exhibited short luteal phases and premature menstruation. Such luteal phase shortening and premature menstruation are consistent with what would be expected as a consequence of diminished secretion of progesterone by the corpus luteum of the ovary. That the analog somehow inhibits the secretion of hormones by the ovary was suggested further by the finding of the consistent premature decline of progesterone and estradiol levels in women who received the analog. This new and unexpected finding of the inhibition of progesterone secretion by an analog of GnRH has two very important implications. First, some types of infertility in women are characterized by conditions of premature menstruation and short luteal phases that are very similar to the premature menstruation and short luteal cycles that can be induced in normally cycling women by the administration of the GnRH analog. Aside from the suggestion that some types of infertility in women may be due to the inappropriate actions of their own GnRH, this new finding affords an opportunity for physicians to develop model systems for use in research programs aimed at the alleviation and cure of certain types of human infertility. Second, this new finding of GnRH analog induced inhibition of progesterone secretion could serve as the basis for the development of the first really new method of fertility regulation in many years. If, as it appears, the conditions induced in women by the GnRH analog are approximately the same as those which occur normally in certain infertile women, postponement of childbearing may be accomplished in women who desire it by the use of GnRH analogs to induce in them a period of temporary infertility akin to that which occurs naturally in certain infertile women.

Pregnancy-Specific Proteins. RSB grantees are working on yet another new and potentially very significant form of fertility regulation of wide applicability. The possible development of a "contraceptive vaccine" has occupied the attention of reproductive scientists for several years. Ideally, the immunity developed through use of such a vaccine would be highly specific and directed against some substance associated only with pregnancy. In the recent past, attention was devoted primarily to human chorionic gonadotropin (hCG) primarily because of the belief that it appears in the body only during pregnancy and is required for the maintenance of pregnancy. At least theoretically then, a woman could be "protected" from pregnancy by treatment with a "vaccine" which would immunize her against her own hCG. Progress in this area of research has been slowed because: 1) since there are apparently other substances in the body that are similar to, if not identical with hCG, the substance may not be "pregnancy specific" as was previously believed; 2) of fear of an increased incidence of abortions and stillbirths due to difficulties in controlling the titer of immunity; and 3) of a generally acknowledged requirement for additional research in reproductive immunology.

Attention has recently been focused on fertility regulation through induction of immunity to the outer envelope of the egg which is known as the zona pellucida. Through the use of sophisticated techniques and instrumentation it is now possible to harvest large numbers of eggs, to separate the zona pellucida from these eggs, and to use such zona pellucida as antigenic stimuli in the induction of immunity to them in animals. Complete and total sterility has been achieved, for short periods, in animals rendered immune to zona pellucida. Since the zona pellucida occurs only on eggs, it appears to satisfy the requirement for pregnancy specificity. However, it is not known precisely what components of the zona pellucida provide the primary antigenic stimuli for the development of immunity. Until these components are identified and other important questions are answered through basic research, the use of this highly promising and apparently highly successful new method of fertility regulation is likely to continue to be restricted to laboratory and domestic animals.

Vitamin A and the Testes. Although there has been much speculation and indirect evidence of a generally accepted relationship between nutrition and reproduction, there are relatively few well-documented reports of a direct relationship between nutrient and a specific reproductive function or process. Our grantees have recently reported such a direct relationship between vitamin A and Sertoli cell function. Sertoli cells comprise a major class of cells in the testis and they play an obligatory, albeit indirect, role in the production of sperm by the testis. One of the known functions of Sertoli cells is the secretion of certain glycoproteins. When Sertoli cells are removed from the testis and grown in long term cell culture (up to four weeks), vitamin A and three other substances (FSH, insulin, testosterone) are required for maintenance of the secretion of the glycoproteins by the cells from the testis.

Vitamin C and Oral Contraceptives. It is known that women taking oral contraceptives (O.C.'s) have altered nutrient metabolism. A decreased level of ascorbic acid (vitamin C) has been implicated as one of the factors possibly responsible for an increased risk of thromoembolism. The mechanism for the

reduced ascorbic acid concentration in women using O.C.'s is unknown but is being investigated in an animal model, the guinea pig. The reduction of tissue levels of ascorbic acid may be caused by a shift of ascorbic acid from one pool to another or may be due to an increase in degradation rates. Preliminary data show an increased pulmonary elimination of labeled carbon dioxide from labeled ascorbic acid, reduced biologic half-life of ascorbic acid, and altered tissue content of ascorbic, dehydroascorbic and diketogulonic acids. These results all indicate that the administration of O.C.'s may promote a more rapid utilization of ascorbic acid and, thus, increase the requirement for the vitamin.

Menopausal Flushes. A close correlation between menopausal flushes and the secretion of luteinizing hormone (LH), the hormone of ovulation, has been revealed in RSB supported investigations. In general, the onset of menopausal flushes is characterized by a sudden intense sensation of facial heat. After spreading to the neck and chest, these sensations extend to the remainder of the body. Although such flushes are experienced by perhaps a majority of postmenopausal women, only vague statements regarding variations in blood vessel muscle activity have been offered in explanation of their etiology. Recent evidence from RSB supported research suggests a close correlation between the intermittant (pulsatile) secretion of LH by the pituitary gland and the occurrance of menopausal flushes. While such flushes did not always accompany LH pulses, on no occasion was a flush observed in the absence of an LH pulse. Since there is evidence to the contrary, the investigators do not claim that menopausal flushes are induced by LH. They suggest instead that both LH secretion and menopausal flushes are initiated by the same neuroendocrine mechanisms that control the precise mechanisms governing the initiation and control of menopausal flushes in women.

Assessment of Sperm Quality. A new assay technique has been developed for monitoring the fertility level of the human male's semen. Men attending a fertility clinic were studied with regard to the physico-chemical state of their sperm quality. Sperm preparations were subjected to controlled denaturation conditions and stained with a dye called acridine orange (AO). The dye fluorescence of individual sperm nuclei was then measured in a cytofluorograf interfaced to a minicomputer. As each sperm nucleus passed through the focus of this cytofluorograf argon laser beam, the fluorescence and light scatter signals generated were measured and reported by the computer. The single AO dye molecules which stick to doublestrand DNA fluoresce a green color, while the multiple AO dye molecules which stackup on groups on a single-strand DNA fluoresce a bright red color. The ratio of red to green color measured by the computer gives an index of DNA denaturation called alpha-t. The work presently reported has shown that abnormal sperm cannot resist denaturation like normal sperm and almost invariably fluoresce a red color. Tests sampled from sub-fertile or questionably fertile animals and men have been found to have higher alpha-t index measurable by the cytofluorograf machine. This work could, therefore, form the basis of a new method of diagnosing certain types of male infertility.

Infertility and Tubal Infections. Scientific clarification of the role of infectious organisms in causing human infertility depends largely on the availability of a suitable animal model for conducting experimental studies. Chlamydiae are currently recognized as among the most common sexually transmitted pathogens and have been implicated as a cause of acute salpingitis. A grantee has recently reported the successful establishment of the first experimental animal model for the infection of fallopian tubes (salpingitis) by Chlamydia organisms. Chlamydia phittaci is a strain of this organisms which causes disease in the guinea pig similar to that in man. It has now been discovered that the inoculation of this organism directly into the fallopian tubes coincident with cyclophosphamide immunosuppression results in severe salpingitis while control inoculation lacking the organism does not. The disease is characterized by an acute and apparently self-limiting course. Signs of inflammation appear two days post-inoculation and peak between 7-10 days. The acute process appears to terminate about 14 days post-infection. Microscopically, the tubes are highly erythematous and a purulent discharge exudes through the fallopian tube opening (fimbria) and coats the ovaries. Ultimately this discharge results in adhesion formations between ovary and tubes and adjacent organs. Histopathological studies indicate that the inflammatory process is largely confined to the tubal surface (epithelial cells) and lumen. Deeper tissue layers are relatively uninvolved. The purulent discharge accumlated in the lumen appears to clear spontaneously and is not found in the lumen 14 days post-infection. The development of this laboratory model will permit important studies to be conducted regarding the effect of these infections on reproductive processes. Many women with salpingitis suffer impaired fertility. It is also possible that Chlamydiae may infect the fertilized embryo or placental tissue in utero with effects potentially as serious as early abortion or fetal wastage. These studies on pathological mechanisms will significantly advance our knowledge of the reproductive consequences of pelvic inflammatory disease.

Inhibition of Ovulation by Prolactin. It has been recognized for some time that hyperprolactinemia and ovulatory failure are highly correlated in women. RSB grantees have recently reported that prolactin inhibits ovulation in the rabbit. These investigators used an in vitro rabbit ovarian preparation which was perfused with hCG alone and in combination with prolactin. While hCG alone promoted ovulation in the preparation, the addition of prolactin resulted in a decrease in the occurrence of ovulation. The effect appeared to be dose related. The precise point at which prolactin interferes with ovulation is difficult to identify. Prolactin may act by interfering with final critical stages of follicular development which ultimately lead to ovulation or with mechanical events within the ovary that are required for rupture of a mature Graafian follicle. These new data provide additional support for the concept that prolactin acts directly on the ovary to influence the process of ovulation.

Monoclonal Antibodies and Sperm Maturation. Monoclonal antibodies produced by fused mutant myeloma cells and spleen cells derived from immunized donors (hybridomas) have recently been used in a study of the process of sperm maturation. Sperm maturation occurs in the epididymis and is required for the acquisition of fertilizing capacity by sperm. Despite its great import-

ance in reproduction, very little is known about those changes that take place in the sperm during residence in the epididymis that render it "mature" and thus, after its capacitation, capable of fertilizing an egg. Through the use of monoclonal antibodies, it has been determined that four new proteins are produced on the surface of the mouse sperm during the process of its maturation in the epididymis. Further studies using this powerful new tool in reproductive biology are likely to clarify additional facets of the important process of sperm maturation.

2. Institutional Programs in Multidisciplinary Reproductive Sciences Research

The RSB supports three types of Institutional Programs in Reproductive Sciences Research: Program Projects, Specialized Reproductive Sciences Research Centers, and Reproductive Sciences Research Centers at leading institutions in the United States. Support for these programs is provided to enable such institutions to develop and to conduct reproductive sciences research programs involving such disciplines and such critical masses of highly skilled scientists as may be required for attacks on complex reproductive sciences problems that are beyond solution by the single investigator working alone or in relative isolation in his discipline. A total of 21 Institutional Programs were supported during the period from October 1, 1979 to September 30, 1980.

A. Program Projects (Funds to support interdisciplinary and coordinated research)

The recipient institutions and principal research areas of the Program Projects supported during the period from October 1979 to October 1980 are: 1) The University of Pennsylvania - biochemical and biological events in reproductive processes prior to implantation; 2) University of Texas at Dallas - neuroendocrine and behavioral interrelationships; 3) University of California at La Jolla - reproductive endocrinology; and 4) Salk Institute for Biological Studies - neuropeptides in reproduction.

B. Reproductive Sciences Centers (Funds to support core facilities within a Center; research funds from other sources)

The recipient institutions and principal research areas of Reproductive Sciences Research Centers supported during the same period are: 1) Vanderbilt University - reproductive physiology and endocrinology; 2) Baylor College of Medicine - mechanism of hormone action; 3) Harvard University - reproductive biology and human reproduction; 4) University of Texas at San Antonio - reproductive mechanisms; and 5) University of California at San Francisco - reproductive endocrinology.

C. Specialized Reproductive Science Research Centers (Funds for research and core facilities for a Center concentrating on topics especially designated by CPR)

The recipient institutes and principal research areas of the Specialized Reproductive Sciences Research Centers supported during the same period

are: 1) The University of Michigan - reproductive endocrinology from molecular to physiological levels; 2) The University of Texas at Austin - control mechanisms at molecular to organismic levels; 3) Case Western Reserve University - reproductive biology of events from ovulation to implantation; 4) University of Texas at Houston - male reproductive function; 5) University of Washington - gamete transport in mammals; 6) Mayo Foundation - mechanism of action and molecular biology of reproductive hormones; 7) Salk Institute for Biological Studies - neuroendocrinology of reproduction; 8) Columbia University- reproductive biochemistry and medicine; 9) University of Pittsburgh - primate reproduction; 10) The Oregon Regional Primate Center - events that control fertility in primates; 11) University of Washington - reproductive medicine and andrology; and 12) The Population Council - reproductive endocrinology.

3. Reproductive Sciences Manpower Development

Effective July 1, 1980, stipend levels were increased for all predoctoral and postdoctoral trainees/fellows who received support under NIH National Research Service Award training grants and individual fellowships. Meeting these new increased annual stipend levels has resulted in a severe curtailment in the funding of new grants. Only by retrenchment in the number of trainees and by severe rebudgeting by the institutions affected has it been possible to maintain the current training programs.

A. Institutional Fellowships

The Institute awards grants for maintenance of institutional environments for research training in the reproductive sciences and for the support of both predoctoral and postdoctoral trainees selected for study in these environments. During fiscal year 1980, NICHD supported 23 reproductive sciences research training grants to 22 institutions. A total of approximately $1,301,000 was used in 1980 by the institutional recipients of research training grants for the support of 95 trainees. Of these trainees, 47 were predoctoral and 48 were postdoctoral fellows.

During this fiscal year a new type of institutional training grant was introduced, the NRSA Professional Student Short-Term Research Training Grant, to expose talented students in health professional schools to the opportunities inherent in a research career. The program is designed to ease the projected shortage of clinical investigators in the future. The trainees under this program have short-term appointments of up to 3 months duration. One such grant was funded with 30 predoctoral students at a cost of $65,124.

B. Individual Postdoctoral Fellowships

In addition to institutional grants for research training, NICHD awards postdoctoral fellowships to individuals for the support of their training in research in the reproductive sciences. During fiscal year 1980, a total of approximately $684,000 was awarded to 36 postdoctoral scientists.

C. Research Career Programs

The Institute awards Research Career Development Awards (RCDA's) to outstanding senior postdoctoral candidates to enable them to devote full time to the development of expertise in research in the reproductive sciences. These awards provide up to $30,000 for salary to recipients for a maximum of five years' support. CPR also supports 1 Research Career Award to an established investigator. Approximately $875,000 was spent in fiscal year 1980 for the support of 23 RCDA's and 1 RCA.

4. Reproductive Sciences Research Facilitation and Augmentation

A. Conferences, Workshops, and Symposia

Conferences, workshops, and symposia are sponsored by RSB, CPR to facilitate the exploitation of new knowledge in critical areas of research in the reproductive sciences and to facilitate the rapid dissemination of new and significant information regarding the reproductive sciences. The following meetings were sponsored during the period from October 1, 1979 to September 30, 1980.

The Annual Meeting of Directors of CPR-supported Reproductive Sciences Centers and Program Projects was held in San Francisco, California, May 1980. It was hosted by Dr. Robert Jaffe, University of California.

Following the Directors' Annual Meeting a workshop on New Horizons in Population Research was held in San Francisco, sponsored by the University of California. Organizers of the meeting were Drs. R. Jaffe and P. K. Siiteri, University of California.

A third workshop on the ovary entitled Physiologic Cessation of Ovarian Function was held in conjunction with the annual meeting of the Society for the Study of Reproduction at the University of Michigan, Ann Arbor, Michigan, August 1980. Dr. Neena B. Schwartz, Northwestern University, chaired the committee that organized the workshop.

A workshop on Animal Fertilization and Embryonic Development In Vitro: State of the Art was held in Bethesda, Maryland, August 1980. The co-chairmen of the workshop were Drs. John D. Biggers, Harvard Medical School and Luigi Mastroianni, Jr., University of Pennsylvania.

CENTER FOR POPULATION RESEARCH

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NICHD Annual Report
October 1, 1979 through September 30, 1980
Office of the Director, Center for Research
for Mothers and Children

The Center for Research for Mothers and Children (CRMC) supports research and research training activities related to the special health problems of mothers and children in the biomedical, social and behavioral sciences. Specifically, CRMC develops and supports basic and clinical research and research training in the biological and behavioral aspects of, and environmental influences upon normal and high-risk pregnancy, embryonic development, fetal growth and maturation, nutritional requirements during pregnancy and infancy, labor and the birth process, postnatal adaptation and maintenance of homeostasis, disorders of infancy, and the effect of perinatal events upon development. The goal is to minimize the risk of morbidity and mortality during the perinatal and infancy period of life.

The Center also supports studies concerned with the precursors of many adult diseases and disabilities, focusing on congenital defects and developmental immunology, nutrition, metabolism, and endocrinology. With these studies, the goal is to prevent chronic disabilities by understanding their genetic, nutritional, metabolic and immunologic antecedents.

The research program of the CRMC includes studies related to the learning process as well as cognitive, personality and social development. Support is given for research that attempts to reveal the nature of the general learning process and certain specific processes such as the acquisition of speech and language and learning to read. Studies of specific learning disorders such as developmental dyslexia are also a major concern. Personality, attitude formation and social development of young children are investigated by a wide range of different scientists such as developmental and experimental psychologists, sociologists, anthropologists, and pediatricians.

In addition, the CRMC has primary responsibility within the National Institute of Health for research and research training in the area of mental retardation. It meets this responsibility through support of a wide range of biological, behavioral, and social research directly concerned with determining the causes and appropriate procedures for the prevention and elimination of the problem. Funds are provided through all of the regular NIH research support mechanisms and through core support to 12 Mental Retardation Research Centers located throughout the country.

In January 1980 after a nearly four-year absence, Dr. Merrill S. Read returned to the NICHD as Chief of the Clinical Nutrition and Early Development Branch and Acting Director of the Center for Research for Mothers and Children. Dr. Read left the NICHD in 1976 to serve as an Advisor in Nutrition Research with the Pan American Health Organization.

In September 1980 Dr. Norman A. Krasnegor, formerly a Research Psychologist with the National Institute on Drug Abuse, joined the Human Learning and Behavior Branch as a Health Scientist Administrator.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Clinical Nutrition and Early Development Branch
Center for Research for Mothers and Children

This is the first annual report for the Clinical Nutrition and Early Development Branch (CNED). The Branch was formed late in 1979 through reorganization of several other branches and programs within CRMC. CNED contains the following sections:

Nutrition and Endocrinology consists of the former Developmental Biology and Nutrition Branch plus selected programs from the Pregnancy and Infancy Branch.

Genetics and Teratology combines the area of congenital defects formerly located in the Pregnancy and Infancy Branch with that of basic molecular and cellular developmental biology previously associated with the Developmental Biology and Nutrition Branch.

Pregnancy and Perinatology contains those aspects of the former Pregnancy and Infancy Branch that are concerned with maternal and fetal health, and newborn well-being, as well as problems of infancy having prenatal antecedents. This Section also is responsible for the NICHD research programs on Sudden Infant Death Syndrome (SIDS). Most of the Major Research Programs (i.e., Perinatology Research "Centers") are managed through the Pregnancy and Perinatology Centers.

The CNED Branch is the largest among the Branches of the CRMC, constituting about 60 percent of the total CRMC budget. Table I summarizes the grant and contract program for each of the three CNED sections in terms of dollars and numbers of awards.

Detailed reports on accomplishments by each of these Sections are presented in the paragraphs which follow.

Table I

NICHD Grants and Contracts, Active During June 1980
Clinical Nutrition and Early Development Branch

(Funds in Thousands)

SECTION	Total		Total Research						Research Training (incl. F32)	
			Total		Grants (incl. RCP's)		Contracts (incl. S06)			
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
TOTAL	697	$63,931	618	$59,917	583	$55,802	35	$4,115	79	$4,014
Nutrition and Endocrinology	172	14,311	154	13,757	145	12,208	9	1,549	18	554
Genetics and Teratology	241	19,853	205	17,703	201	17,273	4	430	36	2,150
Pregnancy and Perinatology	284	29,767	259	28,458	237	26,321	22	2,136	25	1,310

Nutrition and Endocrinology Section

The Nutrition and Endocrinology Section supports research and research training in the following six areas:

1. Developmental Nutrition
2. Infant Nutrition
3. Developmental Gastroenterology
4. Childhood Obesity and Nutritional Antecedents of Adult Disease
5. Cultural and Behavioral Aspects of Nutrition
6. Growth and Growth Factors, including Pediatric Endocrinology

As of July 25, 1980, the Section supported 172 projects at a level of $14,311,000. These projects are analyzed according to programmatic mechanism in Table III.

1. Developmental Nutrition

The Section on Nutritional Endocrinology encourages research directed toward understanding the relationship of nutrition to human developmental processes. The program encompasses studies of nutrition throughout the human developmental period, beginning with nutritional factors relating to pregnancy and fetal development and proceeding through neonatal nutritional adaptation to problems of nutrition and growth during childhood and adolescence.

Presently the three greatest nutritional research challenges of pregnancy are: 1) To assess accurately the nutritional status of a prospective mother; 2) To develop a mechanistic understanding of the total nutritional needs of the human maternal-fetal unit, especially with regard to genetic background and nutritional environment; and 3) To develop appropriate nutritional interventions in order to prevent the birth of physically or mentally damaged offspring.

The Section currently supports research projects that address each of these research challenges. For example, work is progressing on an innovative enzyme-immunoassay procedure designed to assess maternal iron status that avoids the hazards of 125 I radioimmunoassay.

The Section also supports studies of the complex relationship that exists between the mother and her fetus and the means by which nutrients pass from one to the other. Some of this research focuses on the mechanisms of placental transfer of essential nutrients, especially amino acids, calcium, zinc and vitamin A. Research is also supported that is designed to ascertain the effects of excessive or deficient amounts of certain nutrients on the morphologic and endocrinologic development of the fetus.

The Section supports much research on the development of nutritional interventions during pregnancy designed to permit optimal fetal development. Some of this research deals with the possibility of providing intrauterine nutritional therapy, and some of it deals with supplementing malnourished mothers during pregnancy. Encouraging results from one of these latter projects demonstrate that striking reductions can be achieved in neonatal mortality rates by protein

TABLE III
NICHD GRANTS AND CONTRACTS ACTIVE DURING JUNE 1980, BY PROGRAM CATEGORY
Nutrition and Endocrinology Section

Funds (Thousands)

Program Category	Total		Research Grants								National Research Service Awards		Research Contracts (Incl. S06)	
			Total Research		Research Projects		Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	172	$14,311	145	$12,208	130	$10,312	3	$1,461	12	$435	18	$554	9	$1,549
Growth and Growth Factors	72	4,966	58	4,468	53	4,276	-	-	5	192	12	390	2	107
Developmental Gastroenterology	21	1,504	18	1,459	17	1,419	-	-	1	39	3	46	-	-
Developmental Nutrition	38	2,777	32	2,278	30	2,209	-	-	2	69	3	118	3	381
Infant Nutrition	26	3,312	22	2,252	20	1,274	2	978	-	-	-	-	4	1,060
Obesity & Antecedents of Adult Disease	15	1,752	15	1,752	10	1,134	1	483	4	135	-	-	-	-

and caloric supplementation during pregnancy. Offspring of impoverished Colombian women who received an average supplementation of 133 kilocalories and 19.6 gm protein per day during the final 16 weeks of pregnancy experienced a neonatal mortality rate of 22.6/1000. In contrast the rate among offspring of a similar group of women who received no dietary supplement was 44.6/1000.

2. Infant Nutrition

The Section on Nutrition and Endocrinology supports a large program of research on infant feeding. Clinical difficulties associated with the nutritional management of infants born prematurely and of infants who fail to attain normal rates of growth in utero are among the most important research problems of this area.

The 24 research projects that were funded in FY79 as a result of the RFA on Infant Nutrition are already producing interesting and important results. For example, it has now been determined that application of sunflower seed oil to the skin of prematurely born infants can correct their essential fatty acid deficiencies.

Results from research projects that focus on human milk include the discovery of a zinc-binding ligand of low-molecular weight, perhaps citrate, in human milk as well as of enzymes in breast tissue that regulate the triglyceride composition of milk.

The Section supports research on advanced technology to collect, process, separate, store, and deliver human milk and milk components for the clinical care of nutritionally compromised newborn infants. In FY80 it was found that the addition of sodium taurocholate to human milk stabilized milk lipase during heat treatment. This discovery will aid in human milk processing. Methods have also been developed that preserve the immunological functions of human milk; now large numbers of lymphocytes and significant numbers of macrophages can survive storage at -78 C for 24 hours. In a related study of the immunological properties of human milk, it was found that maternal smoking appears to interrupt formation of antibodies against a pool of enteric bacterial antigens.

3. Developmental Gastroenterology

The gastrointestinal tract is the primary interface between the developing infant and its nutritional environment. If, because of disease, congenital defects, or extraordinary metabolic demands, the function of the gastrointestinal tract is impaired, then physiological development will suffer. An important issue which ties these considerations together concerns the effects of human milk on the growth and maturation of gastrointestinal mucosa. For this reason special programming efforts are underway to define growth factors in human milk. As a result of the RFA on Infant Nutrition and the workshop on Gastrointestinal Development held in FY 78, several interesting grant applications received funding in FY 79 and FY 80. These include a project on an epithelial growth factor found in human milk that may turn out to stimulate intestinal development during infancy.

Other newly funded research in the area of developmental gastroenterology concerns studies of intestinal permeability during infancy; the specific functions of bile acids during intestinal development; the sequence of events in the development of pancreatic exocrine digestive function; and a project on breath analysis of carbohydrate absorption in newborn infants.

4. Childhood Obesity and Nutritional Antecedents of Adult Disease

The major objective of this program is to advance our knowledge of the precursors and determinants of obesity. In FY 1980 the NICHD renewed a major commitment to the study of the natural history of obesity by funding the second phase of an analysis of growth and development from infancy to adulthood of approximately 2500 subjects who were enrolled in seven longitudinal studies of growth, some of which were initiated in the early 1930's. Many of the subjects are now being recalled for analysis of body density, serum lipids and fat cell size and number. The NICHD also continues to encourage methodological research concerning obesity and body composition. For example, the development of an air displacement technique for determining body composition of newborn infants is now being perfected. This technique will be used to assess the differences in body composition among newborn infants of various gestational ages, as well as to evaluate degrees of macrosomia in infants of diabetic mothers. Several new grant applications were funded in FY 80 that were submitted in response to a Program Announcement entitled "Overnutrition and Obesity." These new projects include a home-based behavioral program for obese children, another study which analyzes genetic and gestational influences on childhood obesity, and a population-based study on the determinants of continuities and changes in fatness over time.

5. Behavioral and Cultural Aspects of Nutrition

A new program on behavioral and cultural aspects of nutrition was initiated. Five contracts were funded in this area in response to three Requests for Proposals on nutritional individuality. These five contracts focus on food cravings and rejections during pregnancy, various aspects of food allergy, and milk intolerance; the contractors are now completing their first year of data collection.

In FY 80, the Section funded several new grants in the area of cultural and behavioral aspects of nutrition; one on children's conception of food; one on the effect of urbanization and cash-cropping on nutritional status in Kenya; and two on behavioral aspects of obesity.

In June 1979, the NICHD and the NIA cosponsored a workshop on nutrition and behavior which led to the development of a Program Announcement entitled "Developmental Aspects of Behavior and Nutrition" which was published in January, 1980. More than 400 copies of the Program Announcement have been mailed to potential applicants, including the entire membership of the Committee on Nutritional Anthropology. Over 100 inquiries have been received in response to the Program Announcement. By the first deadline of March 1, ten new applications had been received for review, with more having arrived for the July 1 deadline. Two small meetings have been held with experts in nutritional anthropology to discuss further program development in this area.

6. Growth and Growth Factors

This program is concerned with basic and clinical aspects of human growth and development. Although some projects are devoted to somatic growth, skeletal age, and gait development, the majority of the research falls within the areas of developmental endocrinology and neuroendocrinology. Of particular interest are problems of growth hormone deficiency, pubertal onset, and the mechanisms of action of hormones that play directive or permissive roles during human growth and development.

The NICHD currently funds clinical research on endocrine disorders of infancy, childhood, and adolescence, such as congenital adrenal hyperplasia, hypothyroidism, dwarfism, disorders of parathyroid function, abnormalities of sexual development, and disorders of pubertal onset. Exciting advances in this area involve the recent recognition of the heterogeneity of these conditions. For example, about half of the children who fall at or below the third percentile of height for age have normal variant short stature (NVSS). Historically this large group of children has received little attention in terms of biochemical investigation or therapy. New evidence developed by NICHD-supported investigators suggests that about 15% of children with NVSS can be made taller by treatment with exogenous human growth hormone. Even though these children release normal amounts of immunoreactive growth hormone during provocative tests, the hormone appears to be in an abnormally aggregated form, a variant that is biologically less active than normal growth hormone. Another example of heterogeneity involves congenital adrenal hyperplasia (CAH). Although it has been known that several different steroid hydoxylase deficiencies can cause CAH, it has recently been discovered that 21-hydroxylase deficiency can become clinically apparent early in infancy or later in childhood and can be associated with different levels of 17-OH progesterone. The deficient condition has been found to be associated with certain human leukocyte antigens, such as HLA-Bw35, and the gene for 21-hydroxylase also has been localized to human chromosome 6. Information such as this can be useful in detecting CAH prenatally in mothers who have already given birth to one affected infant.

The mystery of the control of puberty continues to be unravelled by several NICHD-supported endocrinologists who have pushed their search far into the brain, moving from the pituitary gland to nuclei deep within the hypothalamus that produce rhythmic electrical impulses that, in turn, initiate pulsed output of LHRH and LH at the time of puberty. This neuroendocrinological cascade of control permits the organism to integrate many different signals that originate from outside and inside the body. These signals relate information about ambient light, nutritional state, levels of other hormones, and even the presence of pheromones in the air. The search for the central origins of puberty serves as a paradigm for future neuroendocrinological studies on the control of parturition, lactation, hunger, satiety, habituation, and nutrient intake.

In FY 80 a randomized, controlled clinical trial of tonsillectomy came to an end after seven years of NICHD support. Over 1400 children were enrolled in the study. By 1978 the investigators were able to report in a lead article in the New England Journal of Medicine 298, 409-413, 1978, that undocumented histories of recurrent throat infection, no matter how impressive, do not

validly forecast subsequent experience, and therefore, do not constitute an adequate basis for operation. The implication is that many children are subjected to needless tonsillectomies. The important new result of the study is that children who meet stringent criteria for operative intervention demonstrate limited but unequivocal superiority of tonsillectomy over non-surgical management in reducing both the frequency and severity of episodes of throat infection over a period of at least two years. During the first year of a randomized trial, only 7% of tonsillectomized subjects experienced three or more episodes of throat infection, as compared with 52% of control subjects. Now that this major question has been answered, the way is paved to answer similar clinical questions involving patients with less severe histories of pharyngitis.

Staff Activities

Dr. Grave represents the NICHD on the following committees: The Interdepartmental Diabetes Mellitus Coordinating Committee; The Nutrition Work Group of the Southeast Asian Refugee Coordination Group; The NIH Diabetes Coordinating Committee; and The NIH Nutrition Coordinating Committee. Dr. Grave also attends meetings of the National Diabetes Advisory Board as an observer.

Dr. Fjellstedt represents the NICHD on The Interdepartmental Digestive Diseases Coordinating Committee, and he serves as an alternate representative to The NIH Nutrition Coordinating Committee. Dr. Fjellstedt also serves as liaison representative to The Commission on Digestive Diseases, to The Committee on Nutrition of the Mother and Preschool Child of the Food and Nutrition Board, and to The Committee on Nutrition of the American Academy of Pediatrics.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title	:	Genetic Linkage Analysis of Juvenile Diabetes
Contract No.	:	NO1-HD-2946
Contractor	:	Regents of the University of Minnesota, Minnesota, Minneapolis, Minnesota, Jose Barbosa, M.D.
Money Allocated	:	FY 80: None as of 8/1/80 FY 79: $263,698 FY 78: $194,542

This comprehensive study focuses on families containing one or more members with insulin-dependent diabetes mellitus (IDDM). The goal is to disclose genetic associations between the juvenile diabetic phenotype and various polymorphic marker alleles that segregate within families of patients with juvenile diabetes. Subjects are tested for 220 genetic markers and several biochemical and histopathological indicators. At this writing these parameters have been determined in 800 members of 136 diabetic families. Certain marker alleles at the B and D loci of the HLA system on human chromosome 6 and the properdin factor B locus on chromosome 6 seem to segregate with a diabetogenic gene or genes. Identification of these markers will provide measures of cumulative risk for individuals who are relatives of insulin-dependent diabetics. Detailed knowledge of the genotypes involved may also allow for identification of environmental factors that interact with susceptibility to trigger the disease. Studies of this nature are the first step in the ultimate prevention of insulin-dependent diabetes by protecting susceptible individuals from potential environmental insults. The aspects of the contract having to do with data collection are nearly complete. A third year of funding is planned for segregation analysis and linkage analysis of the putative IDDM susceptibility gene.

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October 1, 1979 through September 30, 1980
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : Evaluation of Breast Milk for Banking
Contract No. : NO1-HD-2828
Contractor : Baylor College of Medicine, Houston, Texas
Buford Nichols, M.D., and Cutberto Garza, M.D., Ph.D.

Money Allocated : FY 80: None as of this writing
FY 79: $299,662

This contract effort is designed to resolve major problems inherent in the collection, storage, distribution and utilization of banked human milk and colostrum in clinical settings. This is a phased operation in which methods of collection and storage are first being studied systematically. In the second phase the variability of human milk and colostrum will be studied in relation to maternal nutritional status, feeding frequency and other factors. In the third phase the Milk Bank will serve the medical research community by providing well-documented samples of human milk and colostrum and fractions thereof for basic and clinical research. This contract is necessary to place the therapeutic uses of human milk and colostrum on a firm footing.

The workscope for the first year of this contract included evaluation of techniques for collecting human milk, evaluation of various storage containers, evaluation of storage temperatures, evaluation of maternal age and duration of lactation and evaluation of various processing technologies. The workscope for the second year includes evaluation of the effects of heating at various temperatures and under various conditions of light and oxygen on nutrient and immunological components, determination of the nutrient and immunological components of human milk as a function of length of gestation as well as size of the infant, evaluation of the effects of smoking on nutrient and immunological composition of human milk and determination of the nutrient and immunological composition of milk at the time of weaning. The latter three studies will also include determinations of volume of milk produced. In addition, two methods for preserving the cells of human milk will be evaluated. Studies of the apparent decreased adherence of cellular components of milk are proposed, including preliminary studies to determine the nature of the supernatant factor thought to be reponsible for the decreased adherence. Important studies to determine the effects of mixing milks from different donors are also proposed. Other processing technologies will also be evaluated, including high temperature processing, fractionation, and freeze drying.

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October 1, 1979 through September 30, 1980
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : Mechanisms of Food Protein Intolerance
Contract No. : NO1-HD-2834
Contractor : University of Texas Medical Branch, Galveston, Texas
Geraldine K. Powell, M.D.
Money Allocated : FY 80: None
FY 79: $282,564 for 3 years

Project Aims: 1) to quantitate macromolecular absorption and examine some mechanisms of its control; 2) to examine the role of sensitized lymphocytes in food intolerance.

Progress to Date: Antigen challenge studies have been completed on eighteen infants with egg protein, soy protein and bovine casein. So far 30 challenges were negative, 18 were positive, and 12 were equivocal.

Lymphocyte transformation was studied in regard to these reactions. Lymphocyte stimulation indices were found to be greatest with soy protein challenges and least with egg protein challenges.

Serum antibody studies are in progress and titers of IgA and IgM are being analyzed with respect to egg, milk and soy antigens.

Work is also in progress on eliminating the interference of serum antibodies in the assay of ovalbumin antigen. The most promising approach seems to be the use of gel filtration to separate gamma globulin from the ovalbumin.

In the coming year the contractors plan to continue these observations and analyses on a larger number of infants.

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October 1, 1979 through September 30, 1980
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : Dietary Cravings and Aversions During Pregnancy
Contract No. : NO1-HD-2837
Contractor : Albany Medical College, Albany, New York
Ernest B. Hook, M.D.
Money Allocated : FY 80: None

Project Aims: An epidemiologic study designed to characterize several factors relating to dietary cravings and aversions during pregnancy. Among these are: 1) the type and frequency of cravings and aversions; 2) the reaction to food as a function of dietary habits prior to pregnancy; 3) the association of cravings and aversions with other biological and demographic factors; 4) the time during gestation when cravings and aversions occur and establishment of recognizable trends over the course of gestation; 5) the changes in maternal diet and in the rate of exposure to embryo toxins such as tobacco smoke and alcohol associated with cravings and aversions; and 6) the association of cravings and aversions with fetal outcome.

Progress to Date: Four hundred and fifteen subjects have been interviewed so far. A complete coding scheme for the data, which involves over 1100 variables per subject, has been devised. The coded information has been entered into machine usable form directly to magnetic tape, bypassing the usual card punch method.

Error checking and editing of the data set has begun, and several sub-sets have been established to ease this process.

Preliminary descriptive statistical tables of 70 categories of cravings and aversions experienced during the pregnancies of the 415 subjects have been prepared. These include a tabulated analysis of the reasons given for each of the cravings or averions.

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October 1, 1979 through September 30, 1980
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title	:	Studies on Development of Food Allergy in Infants and Children
Contract No.	:	NO1-HD-2833
Contractor	:	University of California, School of Medicine, San Francisco, California, Oscar L. Frick, M.D., Ph.D.
Money Allocated	:	FY 80: None FY 79: $294,348 for 3 years

Project Aims: To study the natural history of the development of food allergy in infants in order to discover what circumstances generate allergic sensitization to foods.

In pre-school children allergy to certain foods accounts for one-third of all allergic problems, and one percent of all babies born experience allergic reaction to cows' milk. Allergy to cows' milk is associated with a four-fold increase in the incidence of asthma and allergic disease later in life. In order to determine the natural history of food allergy and the frequency of immunological reactions associated with its development, the contractors will follow from birth a group of 24 infants born into bilaterally allergic families. It is expected that at least half of these infants will develop food allergies within their first year of life.

Progress to Date: Several babies have now been enrolled in the study none of whom has yet shown any signs of allergy.

The contractors have also performed leukocyte inhibition assays (LIF), lymphoblastogenesis tests, and Raji tests for circulating immune complexes in 15 children and 3 adults with documented food allergies. The LIF tests were positive for one or more foods in 13 of the children and in the 3 adults. The Raji test was markedly positive in 3 of 3 tested. The lymphoblastogenesis test was less satisfactory (positive in 6 of 14 tested).

Problems encountered: The number of clinic patients being seen has dropped due to drastic reductions in clinic personnel necessitated by the fiscal constraints imposed by the passage of Proposition 13. Patients will now have to be recruited from other hospitals which will require more reviews by Human Experimentation Committees.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : The Prevalence of Lactose (Milk) Intolerance and its Impact on Vitamin-D Status in Canadian Indian and Inuit Children
Contract No. : NO1-HD-2844
Contractor : University of Western Ontario, London, Ontario
J.J. Ellestad-Sayed, Ph.D.
Money Allocated : FY 80: None
FY 79: $183,770 for 3 years

Project Aims: Lactose malabsorption is common among the Inuit (Eskimos) and Indians of Canada (73% and 60%, respectively). This study proposes to determine the prevalence of milk intolerance in these groups and relate it to the effectiveness of the vitamin D milk fortification program in Canada.

Progress to Date: Children who are lactase-deficient will be identified by increased concentrations of hydrogen in their breath after ingesting milk or lactose. The contractors have constructed an apparatus for collecting breath from preschoolers and this instrument is now being tested. Optimal conditions for detection of breath hydrogen have been determined and hydrogen standards from 6-50 parts per million have been found to be detectable in a linear manner.

The contractors have also developed an assay for 25-OH Vitamin D. Recovery of 25-OH Vitamin D from the extraction procedure has been increased from 84% to 96%. Trial runs of the complete method show that as little as 10 picograms of 25-OH Vitamin D can now be detected.

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October 1, 1979 through September 30, 1980
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title	:	A Prototype Allergic Disease Prevention Program: Clinical and Immunological Consequences
Contract No.	:	NO1-HD-2832
Contractor	:	University of California, School of Medicine, San Diego, California, Robert S. Zeiger, M.D., Ph.D.
Money Allocated	:	FY 80: None FY 79: $327,199 for 2 years

Project Aims: To document by modern immunologic methods that pre- and postnatal dietary and environmental manipulations lead to the reduction of immediate sensitization and subsequent development of allergic disorders secondary to such sensitization.

Progress to Date: Approximately 100 positive allergic families have been entered in the San Diego experimental group. The ongoing rate of enrollment is now about 30 families per month in this group. The control group has approximately 139 families from San Diego and Bellflower Hospital currently enrolled. Because the families are enrolled while the mother is early in pregnancy, the experimental group now has 25 live births (with cord blood) and the control group has 20 live births (also with cord blood). These numbers will increase rapidly now that there is a steady flow of families enrolled. Family history data and pertinent medical data for all families involved in the study are immediately computerized in preparation for preliminary statistical analysis.

Genetics and Teratology Section

The formation of the Genetics and Teratology Section strengthens the NICHD emphasis on studies of the biological development of the human organism and focuses on congenital defects as a research priority area. This Section supports research on the underlying mechanisms controlling both normal and abnormal development. To foster the necessary studies, the Section is structured around the following areas: genetics; congenital defects; and developmental immunology.

GENETICS
Biochemical and Molecular Genetics

Of particular interest to studies of normal and abnormal development is the control of expression of genes. Several genetic regulatory mechanisms are currently being investigated.

One of these mechanisms is the control of the differential functions of genes. A number of studies probe into the expression of "structural" genes which code for specific gene products (proteins) and emphasize their control by nearby "temporal" and "spatial" regulatory genes. Other investigations address the control of genes by components of the genome other than adjacent sequences of DNA. The mechanism by which either flanking DNA sequences or various non-histone chromosomal proteins promote selective expression of genes is barely beginning to be understood.

The mechanism of selective gene function is but one consideration in the regulation of expression of genes. Another mechanism is suggested by the fact that eukaryotic cells synthesize a much larger amount of RNA in their nucleus than is exported to the cytoplasm for the purpose of producing a specific end-product protein. The role of the high-molecular weight nuclear RNA, designated HnRNA, as the precursor of cytoplasmic RNA messenger, and its processing into mRNA, is currently being investigated. Furthermore, mRNA contains a large homopolynucleotide tract of adenylic acid residues, poly(A), which is being studied. The function of poly(A) in processing RNA molecules and thus in regulation of gene expression still must be learned.

A third mechanism for control of gene expression is utilization of cytoplasmic RNA in protein synthesis. This process is being investigated with emphasis on the usage of prefabricated mRNA immediately after fertilization for ribosome interaction, and the role of messenger-and tissue-specific initiation factors in stimulating the mRNA-ribosome association. Specific factors which are inhibitory for protein synthesis are also being studied. These investigations are directed towards demonstration of the existence of a translational control mechanism as one of several regulatory processes for the expression of genes.

Developmental Genetics

Developmental genetics covers a broad area including molecular control of gene expression as well as organogenesis and its genetic control. Studies

include the identification and subsequent analysis of (1) genes concerned with processes of development, (2) mechanisms of the gene action, and (3) mechanisms of development.

The developmentally important process of cell determination gradually restricts the multiple potential of cells until they are capable of only one type of genetic expression. During this process, particular cell types develop a mechanism to select sequences of DNA for purposes of expression while the rest of the DNA sequences remain repressed. The molecular events which bring about determination remain to be defined.

The genetic basis of determination is currently under investigation using mutants to identify the genes operating in the normal determination process. The investigations also explore the genetic basis of cell interactions since post-determinative differentiation in later organogenesis is specified by the interactive events. Other epigenetic factors in determination and early development are also under study. Cytoplasmic substances that have been synthesized during oogenesis are investigated for their role in the early developmental process. Attempts are made to identify the genes specifying the cytoplasmic products which influence early development, to establish the identity and function of cytoplasmic molecules (RNAs or proteins) in the epigenetic regulation of gene function, and to determine the mechanism of localization of the epigenetic control substances in the cytoplasmic matrix. These studies are directed at problems of whether maternal cytoplasmic substances contain information which specifies organization of the fetus during later developmental stages.

During the second developmentally important process of cell differentiation, cells that have undergone determination express their unique genetic potential to become specialized cells of specific tissues and organs. Differentiation is influenced by cellular interactions as well as by the timing of the developmental event. The different factors which contribute to the differentiation process during the formation of organs, as well as the interdependence of these influences, are currently under investigation with particular emphasis on development of the limb. Studies of the limb include factors influencing muscle, cartilage and bone differentiation. Investigations emphasize localization of muscle genes on chromosomes and identification of structural muscle genes (as well as skeletal genes) affecting limb shape. They further stress the structure of collagen genes and collagen gene function as well as structure and function of adjacent regulatory genes. The various processes by which limb bud (and for that matter other organ) cells sort themselves out and interact for differentiation at specific developmental times into special cell types need continued investigation.

Classical twin studies are being used to investigate the causes of variations in genetic traits. A half-sib model is being used to analyze data from the spouses and offspring of monozygotic twins as well as from the twins themselves. This clinical model allows an estimation of many important genetic and environmental parameters as well as the evaluation of maternal factors when kinships of male and female twins are contrasted.

Further study will focus on birth defects, causes of fetal wastage, normal growth and development, and psychological traits.

Remarkable advances are also being made in understanding gene control of mammalian development. Unique mouse models with mutant cell lines which contain defects found in humans have been developed for studies of human genetic disease. These animal models hold great promise for the future study of human genetic disease as well as of normal and abnormal development.

Cytogenetics

Cytogenetics analyses include assortment and breakage of chromosomes with emphasis on translocation, duplication or inversion of genes in early gametogenesis. Other studies emphasize structural and functional changes of sex and autosomal chromosomes, beginning during late spermatogenesis, and alterations in the number of chromosomes as a result of chromosome or genome inactivation during the spermatogenic developmental event.

CONGENITAL DEFECTS

A major NICHD objective is to expand the emphasis on prevention of congenital defects in children by encouraging studies of factors that adversely affect growth and development of the conceptus, embryo, fetus and child. Techniques and knowledge of cell biology and developmental genetics are utilized in combination with clinical and epidemiological studies. Temporal and spatial factors as well as the ever changing internal environment within the maturing organism affect the function of genes and their products.

Another task is to identify the response of specific human genetic constitutions to particular biological and behavioral environments. Both external the physical environment as well as the intrauterine environment must be emphasized in solving the problems.

The role of hereditary and environmental factors in abnormal development is now beginning to be studied in the limb bud. This system is used since its normal developmental processes (some of which were outlined above) are actively under investigation and since the limb is especially sensitive to teratogenic influences, particularly to environmental agents as illustrated by the thalidamide tragedy more than ten years ago. Other systems studied are the lung and brain. In all three instances, the emphasis is on genetic and environmentally-produced teratogenic aberrations of morphogenesis, biosyntheses, and interdependence of developmental processes which could lead to abnormalities of these developing organs or body structures.

DEVELOPMENTAL IMMUNOLOGY

Ontogeny and Phylogeny of Immunity

The NICHD has developed a research program that explores the development of the immunologic system, beginning with the intrauterine period and extending through full adult immunologic maturation. The focus on the ontogeny of immunity is supplemented with studies of the phylogeny of immunity. These

studies provide insights into the normal evolution of the reticulo-endothelial system and assist in the selection of lower animal models that allow research not feasible in the human. Other studies in both human and animal models are concerned with immunodeficiencies, their causes, and the development of new therapies. These studies will provide baseline information on precise aspects of the developing immune system at various maturational periods in development, will lead to an understanding of the mechanisms controlling these functions, and will identify the aberrations from the normal leading to infection and disease.

Newborn Infections

Of special concern is the immunologic vulnerability of prematures and newborns resulting in increased morbidity and mortality due to infections, e.g., viruses, gram negative bacilli, and Group B streptococci. Studies include both cellular and humoral aspects of the defense system with an attempt to investigate the characteristics found in the premature and newborn that make this developmental period more susceptible to these infections. Study of the ontogeny of these events should produce important insights into the prevention and management of intra-uterine infections and their resultant sequelae such as growth dysfunctions, mental retardation, and congenital malformations.

Immunology of Breast Milk

Breast milk contains both nutritional and immunologic factors that have been discovered recently. Therefore, breast feeding is being evaluated for the beneficial effects it may provide all infants, but especially to the high risk infant in whom either the nutritional and/or immunologic imbalance may be precarious.

Studies include exploration of the immunologic properties of breast milk, the mechanisms that may pertain to the mother's experiences with past infections, the transfer of protection to the infant through breast-feeding, and the events that occur when the ingested milk encounters the infant's digestive tract. Information on potential adverse effects of breast feeding will also be pursued. A publication, entitled Immunology of Breast Milk has resulted from an NICHD sponsored conference to stimulate further research in these areas.

The gastrointestinal tract is often the portal of entry for agents causing neonatal sepsis. Therefore, NICHD has expanded its research activities to better understand regional immunity mediated by locally formed antibody and viable immunologic cells and other factors associated with mucous membranes exposed to the external environment. A broadened understanding of the local defense mechanisms of the gastrointestinal tract are important in studies of the immunology of breast milk.

Reproductive Immunology

The mammalian fetus generally survives pregnancy even though it contains some characteristics which are foreign to the mother's immune system. The fetus

becomes an allograft to the mother during pregnancy and successfully maintains a close association with the mother markedly exceeding the normal rejection time of most allografts. The means by which the fetus is tolerated by the mother is not adequately understood. Thus, if the ontogeny of immunity is to be fully appreciated, the fetal period must be properly evaluated in regard to the immune response to this fetoplacental unit and the maintenance of pregnancy.

STAFF ACTIVITIES

A staff member of this Section has been appointed to the NIH Cystic Fibrosis Coordinating Committee which functions to stimulate and plan for research activities across NIH. Staff also participates on the NIH Coordinating Committee for Blood Related Activities and the NIH Working Group For Reye's Syndrome. This later group is planning for collaborative research funding between Institutes and is assisting in the development of plans for a Concensus Development Meeting for Reye's Syndrome. A member of the Section has also been appointed to the Genetics Education Committee of the Health Services Administration (HSA). This committee is developing recommendations for the improvement of genetics education in the HSA funded State Grant Programs providing Genetic Services across the nation.

NICHD GRANTS AND CONTRACTS, ACTIVE DURING JULY 1970
Genetics and Teratology Section

Funds (thousands)

Health Area	Total		Total Research						Fellowships (incl. T32)	
			Total		Grants (incl. RCDs)		Contracts (incl. SCs)			
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	239	$20,291	208	$13,230	205	$17,[illegible]	3	$276	31	$2,[illegible]
Cellular and Organ Differentiation	67	5,424	54	4,161	53	4,113	1	47	13	1,263
Developmental Genetics	50	4,617	43	4,443	42	4,179	1	264	7	174
Developmental Immunology	57	4,420	52	4,283	51	4,213	1	65	5	136
Developmental Pharmacology	3	171	3	171	3	171	-	-	-	-
Congenital Malformations	62	5,660	56	5,171	56	5,371	-	-	6	488

Notes: (1) Excludes one scientific evaluation grant for $[illegible].
(2) Excludes two contracts for $135,263 funded from a source other than NICHD.

Pregnancy and Perinatology Section

The Pregnancy and Perinatology Section supports and promotes a coordinated program of research and research training to enhance advancement of knowledge related to pregnancy and maternal health, fetal growth and maturation, and newborn well being. Activities evolve around five maternal-infant problem-oriented health areas: High Risk Pregnancies; Fetal Pathophysiology; Premature Labor and Birth; Disorders of the Newborn; and the Sudden Infant Death Syndrome.

Program goals recognize the interrelationships of specific health and developmental problems occurring in the prenatal, perinatal, and infant periods of life and the effects that these events may have upon development and well being of the child. Particularly relevant are maternal health problems which affect fetal and infant health, problems with which the newly born infant must cope in adapting to extrauterine life, and events occurring during the neonatal period which can influence the development of the baby.

Section-supported research activities are concerned with the physiology and complications of pregnancy, placental functions, lactation, developmental biology, processes of embryonic development and fetal growth, antenatal and intrapartum diagnostic procedures, the process of labor and delivery, and diverse environmental factors which can affect the pregnant woman and her fetus. Areas of research endeavor related to the newborn infant include respiratory function, metabolic processes and related problems, heat production and temperature regulation, infectious diseases, and the sudden infant death syndrome.

The Pregnancy and Perinatology Section is responsible for the administration of approximately 28 percent of the Institute's Center for Research for Mothers and Children extramural holdings, numbering 283 grants and contracts and amounting to approximately 30 million dollars. An estimated 4 percent of the Branch's total budget expenditures provide research training support in areas pertinent to pregnancy and maternal health, developmental biology, and perinatology.

An estimated 72 percent of the Section's research project funds provide support for studies pertaining to High Risk Pregnancy (31%), Fetal Pathophysiology (25%), and Disorders of the Newborn (16%); fifteen percent (15%) for research specifically on the Sudden Infant Death Syndrome, and thirteen percent (13%) to studies of Premature Labor and Birth. Program holdings for each area by support mechanism appear in Table IV.*

*The above figures were based on active June 1980 for CNPP.

TABLE IV

NICHD GRANTS AND CONTRACTS, ACTIVE DURING JUNE 1980

Pregnancy and Perinatology Section

Funds in Thousands

Health Area	Total		Total Research						Fellowships (incl. T32)	
			Total		Grants (incl. RCP's)		Contracts (incl. S06)			
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	283	$29,742	258	$28,433	236	$26,296	22	$2,136	25	$1,310
High Risk Pregnancy	88	9,079	80	8,782	77	8,477	3	305	8	297
Fetal Pathophysiology	62	7,445	55	7,115	54	7,078	1	38	7	329
Premature Labor and Birth	38	3,874	38	3,874	36	3,840	2	35	-	-
Disorders of the Newborn	64	4,737	54	4,054	48	3,875	6	179	10	683
Sudden Infant Death Syndrome (Primary)	31	4,607	31	4,607	21	3,028	10	1,580	-	-

Note: A scientific evaluation grant for $25,000 is excluded.

NICHD-OPE-PAS
July 14, 1980

Research Accomplishments

Selected scientific accomplishments during FY 80 pertinent to the Section's five problem oriented health areas are highlighted in the following paragraphs:

Researchers are examining the mechanisms by which progesterone acts as a powerful anti-inflammatory-immunosuppressive steroid when released in tissues, and the mechanisms that regulate placental progesterone synthesis. These studies found that a sharp decline in the production of progesterone precedes parturition in most mammalian species, but this has not been clearly established in the human. It was found that fetal pregnenolone is an important precursor of placental progesterone. Furthermore fetal cortisol secretion both decreases pregnenolone secretion and inhibits its conversion to progesterone, thus providing a mechanism for a 30-40 percent reduction in placental production of progesterone that may go undetected in the maternal circulation. Since it is known that progesterone production is decreased about fifty percent in women who undergo premature birth, these data suggest that a similar reduction may occur before normal birth, and that increasing placental production by infusion of a precursor may be a way to prevent premature birth.

Studies are trying to delineate and compare the endocrine-metabolic control of glucose homeostasis and insulin resistance in pregnant women with diabetes receiving either the American Diabetes Association Diet (ADA) or a high carbohydrate-high-fiber diet (HCF). A special HCF diet has been developed for use in pregnancy; clinical studies to date suggest that its use results in smoother, less erratic glucose control. Laboratory studies of pregnant diabetics indicated that a 26% decrease in maximal insulin binding to erythrocytes occurs during the late stages of pregnancy. The decrease is due to a 40% reduction in receptor concentration rather than a change in binding affinity. Such decrease in binding occurs without a significant difference in fasting insulin levels between pregnant and nonpregnant control subjects. However, during the first 24 hours postpartum, binding increases to nonpregnant levels in association with an increase in receptor affinity. These findings are significant because of both their health implication and the possibility of translating new laboratory observations directly into improved patient care for a chronic metabolic problem that has long range importance for diabetic mothers and their infants. These findings also suggest that a change in dietary management for pregnant diabetics, coupled with a more quantitative outpatient approach to monitoring insulin dosage, results in better control of plasma glucose values, and may improve pregnancy outcome.

Investigators are developing methods to predict fetal volume and weight by utilizing ultrasonic scans. They have so far developed a computer system to calculate fetal and organ volumes. The system will enable prediction of fetal size and weight which are important indicators of fetal well-being. The detection of intrauterine growth retardation (IUGR) will enable the institution of therapeutic regimes for the successful management of IUGR and reduce perinatal morbidity and mortality.

Ongoing research is studying the factors which affect placental respiratory gas exchange, those which control fetal cardiac output, and the influences on

oxygen diffusion and utilization in fetal tissues. For the first time the fetal dose-response relation for norepinephrine has been measured as well as the resulting increase in fetal O_2 consumption. Even with a 25% increase in O_2 consumption, fetal blood gas levels were unchanged. This indicated that the placental reserve function exceeded this increase in fetal oxygen utilization. Also, in conditions of fetal hypoxia blood is shunted preferentially to the brain, but not to any particular brain region. Cerebral blood flow to all regions varied inversely as a function of O_2 tension and directly as a function of CO_2 tension and hydrogen ion concentration. Investigators also studied the effects of maternal exercise (and possible resulting fetal hypoxia) during pregnancy. In guinea pigs exercised 15, 30, 45, or 60 minutes daily throughout pregnancy, fetal and placental weight varied inversely with exercise, and placental diffusing capacity decreased significantly in the 15 to 45 minute exercise groups. The various studies completed or projected may help in elucidating the role of the numerous factors in maintaining optimal O_2 delivery to fetal tissues.

Studies of rat brain and spinal cord are trying to determine what parts of the oxytocin system are involved in neurosecretion and their regulation. From the immunocytochemical data obtained, it is believed that oxytocin in the brain has many roles in addition to the recognized one related to suckling uterine contraction. Innervative connections found to the cerebral cortex imply some participation in more sophisticated integrative activity. Additional connections found in the hypothalamic brain with other sites via oxytocin fiber projections, probably from the paraventricular nucleus, suggest roles in pain and the regulation of blood pressure.

In the area of initiation of labor, studies to clarify the precise mode of action of various hormonal stimuli on the cellular and subcellular level in the uterus are continuing. Research work is examining subcellular organelles, enzymes and proteins involved in myometrial calcium transport and interactions. The investigation involves the purification and characterization of bovine sarcoplasmic reticulum, cell membrane and bovine Ca, Mg-ATPase and Ca-binding proteins. To date it has been possible to purify a 45,000 molecular weight calcium binding protein from the deoxycholate extract of sarcoplasmic reticulum by column chromatography. The findings can contribute to a better understanding of the factors involved in the onset of labor and in the treatment of insufficient, dystocic, or premature labor.

Studies in progress are attempting to determine if transfusion-acquired cytomegalovirus (CMV) infections in newborn infants can be prevented by using blood donors who have no serological evidence of having had a CMV infection in the past. In studies of infants of seronegative mothers, 13.5% of infants who received transfusions from seropositive donors became infected, compared to none receiving transfusions from seronegative donors. A process to simplify screening of blood for transfusing newborns has been developed The studies are currently directed toward determining whether or not it is necessary to screen plasma donors. If it proves unnecessary to screen plasma donors, implementation of donor selection programs for nurseries would be simplified.

Important research is addressing the problem of temperature regulation in premature infants and the mechanisms responsible for endogenous thermogenesis. Studies are looking at the developmental changes in cellular respiration of brown adipose tissue during fetal and neonatal development and the influence of thyroid status on these parameters. The studies suggest that the premature infant may have immature cellular function of the brown adipose tissue which interferes with the ability to increase endogenous heat production during cold stress. This immaturity seems associated with a decrease in mitochondrial enzyme activity. These studies determined that thyroid hormones are not necessary for thermogenesis in premature infants.

The synaptic development in three specific regions of the rat brain stem is being studied. It was found that the most rapid period of synaptic development occurs during the early postnatal period, a period relevant to the sudden infant death syndrome (SIDS). The period of most rapid synaptic development may be the period when overt external forces will most effectively modify its morphology and function. The correlation of SIDS with maternal smoking suggests that induced hypoxia can be one important external factor that modifies this synaptic development.

Comparative physiologic techniques are being developed to study fetal and neonatal metabolism, for example, fetal lambs and guinea pigs were infused with 14-C-lysine to study protein synthesis and turnover, its rate of metabolism, and its role in fetal carbohydrate metabolism. Useful data have been obtained concerning the role of free fatty acids, ketoacids and acetate as potential alternate fuels for the placenta under conditions of maternal fasting. In addition, a number of smaller peptides were identified which are contaminants of commercial HCG. One of these contaminants is an antitrypsin; its relationship to HCG and its potential role in blocking HCG antibodies are being studied. Investigators are also studying intraventricular hemorrhage in pre-term high-risk infants, comparing the diagnostic accuracy of ultrasound and CAT scans in this condition, and performing serial coagulation studies to clarify the role of clotting disorders in causing intracranial hemorrhage.

Studies are trying to determine whether survivors of infant respiratory distress syndrome (RDS) subsequently have greater respiratory disease morbidity than control infants, and to resolve whether the organisms responsible for respiratory infections are different for RDS survivors than for control infants. Initial findings suggest that the rate of all respiratory illnesses is not different, but the quality of infection may be dissimilar with more lower respiratory illness in the RDS infants. Assessment of risks related to gestational age groups, to presence or absence of RDS,to passively acquired antibodies, or to breast feeding may allow the selection of high-risk patients for the evaluation of various therapeutic modalities.

Major Research Programs

Major Research Programs (MRP) are program grants which promote and support multidisciplinary research efforts to develop new knowledge about diseases and disorders of pregnancy and neonate. The Pregnancy and Perinatology Section is participating in the Institute's Major Research Program activities. It is responsible for the administration of six of the seven MRPs. Three of the MRPs are concerned with the maternal-infant health problem of diabetes mellitus in pregnancy. Other MRPs are involved with investigating the problems of: (1) the sudden infant death syndrome; (2) prematurity and the initiation of human parturition; and (3) maternal smoking, fetal hypoxia, and neonatal outcome.

Dr. N. Freinkel and his colleagues at Northwestern University Medical School are investigating diabetes mellitus during pregnancy, including the events prior to, during and after delivery as they affect the infant and mother. The major thrust is to develop proper clinical management of the disease. Studies on hemoglobin A1C and multiple metabolic parameters have disclosed significant differences in maternal Hg A1C values obtained in the third trimester in those pregnancies associated with macrosomic infant versus those in which macrosomia was not found. Studies on receptors for glucoregulatory hormones in pregnancy reveal that post-receptor phenomena may be of major significance in the insulin resistance observed in late gestation.

Dr. William Oh and team at Brown University are continuing their multidisciplinary, multifaceted examination of the effects of diabetes in the pregnant woman and her offspring. They found that hyperviscosity influences glucose homeostasis, with lowering of plasma glucose value, but the mechanism is not related to insulin release. From studies on obese Zucker rats, these investigators found that fat cells do not contribute to insulin resistance; the soleus muscle metabolism is defective in that a marked decrease occurs in cell surface receptors for insulin. They pointed out that the effects of bilirubin on fat cells metabolism are unrelated to competition between bilirubin and fatty acids for protein binding sites. Potentially important data has been obtained in the study of adenine nucleotide levels in erythrocytes from diabetic and normal mothers and their neonates. From measures of visual exploration, results show reliable behavioral differences which are relatively stable between 2 and 4 months of age.

Drs. M. Rosen and S. C. Kalhan, collaborators at the Case Western Reserve University, are studying the disorders in diabetic pregnancies where a surfeit or deficiency of substrate *in utero* occurs causing either morbidity in the perinatal period, or aberrations of neurological and cognitive outcome persisting throughout life. They indicated that epinephrine deficiency was the most specific finding among abnormal children. This observation supports the hypothesis that severe perinatal stress may result in persistent injury to the adrenal medulla. Measurement of fetoplacental steroid metabolism was found to provide a reliable assessment of fetoplacental function and thereby may have predictive value for fetal outcome. In addition, the finding that visual neurological systems are capable of some degree of discriminative function as early as 32-34 weeks from conception constitutes an important contribution.

Dr. A. Steinschneider and collaborators at the University of Maryland are conducting prospective studies evaluating the validity of a variety of proposed neonatal indicators of risk status for sudden infant death syndrome (SIDS). Results so far indicate that predisposed infants pass through an age-related stage wherein prolonged sleep apnea can occur during a nasopharyngitis but not when free of illness. At still a later age, episodes do not occur even when the infant has a nasopharyngitis. These data suggest that infants who die of SIDS in association with clinical evidence of nasopharyngitis would be in general older than those who were not ill when death occurred. Studies are in progress which will permit the simultaneous examination of several other etiologic hypotheses and suggested approaches for risk identification.

At the University of Texas, Dr. P.C. MacDonald and collaborators have studied the physiological basis of initiation of labor as means of prevention of prematurity. They discovered that luteinizing hormone releasing hormone (LHRH) and thyrotropin releasing hormone (TRH) in human hypothalamic homogenates are present in separate sets of subcellular particles which have physical properties similar to isolated nerve terminals, i.e., synaptosomes. They found that lipoproteins are the principal sources of cholesterol used by the human fetal adrenal (HFA) for steroidogenesis and low density lipoprotein (LDL) is the preferred lipoprotein. The uptake and degradation of LDL by normal trophoblastic cells is mediated by a high affinity, low capacity plasma membrane receptor. From studies on amniotic lysosomes, these investigators found that an alteration in their physiology is an important step for the initiation of human parturition.

Dr. L. S. James and a group of investigators at Columbia University Presbyterian Medical Center are studying the characteristics of fetal breathing and fetal heart rate patterns and their relationships to gestational age or to fetal hypoxia. They are trying to determine if the antipartum patterns relate to intrapartum patterns and correlate with intrapartum high risk prediction indices. In addition, they are studying whether maternal smoking may cause fetal hypoxia. Research is in progress in its first year of investigation. Data for three regression analyses have been collected to clarify the complex interrelationships between intrauterine growth retardation, intrapartum hypoxia, smoking, neonatal neurologic outcome, and poor maternal weight gain This research will provide new information on human fetal responses and permit the assessment of the condition of the fetus and facilitate the prediction of pregnancy outcome.

Sudden Infant Death Syndrome

During FY 1980, the Pregnancy and Perinatology Section continued to strengthen the Institute's sudden infant death syndrome (SIDS) program. This program is a broadly based research effort that aims to understand the underlying mechanisms of the syndrome, to determine its etiology, to identify infants at risk, to explore preventive approaches, to inform the scientific and lay communities about SIDS, and to stimulate scientists to direct their investigative efforts to the solution of this complex biomedical problem.

The Institute's seven SIDS research emphasis areas continue to be the focus of its investigative effort. These areas include: (1) developmental neurophysiology with emphasis on sleep patterns and associated autonomic disturbances; (2) respiratory, cardiac, and laryngeal responses to specific stimuli; (3) metabolic, thermal, and endocrine factors; (4) perinatal development of immune response mechanisms and infectious disease processes; (5) psychological stresses experienced by parents and siblings when a sudden, unexpected, and unexplained infant death occurs; (6) epidemiologic, environmental, and genetic aspects, and (7) anatomic pathology.

The 96th Congress passed PL. 96-142-Emergency Medical Services Systems amendments which continues the requirement that the Secretary, Department of Health and Human Services, provide adequate funding for NICHD research activities in SIDS. In fiscal year 1979, the latest year for which an annual total is available, the Institute obligated $11.1 million to support 105 research projects. of this total, $4.9 million supported 38 research projects specifically concerned with SIDS. The remaining $6.2 million provided support for 67 projects generally related to the problem of SIDS.

As a result of Institute supported investigations of SIDS since 1972, there is increasing support for the idea that the victims of crib death are not completely healthy and their deaths not as inexplicable as once thought. Many of these infants have subtle physiologic defects of a neurologic, cardiorespiratory, and/or metabolic nature which may reflect delays or changes in the victim's maturational processes. Moreover, there is increasing evidence that the pathogenesis of the syndrome is not through a single mechanism as previously believed. Indications that these infants have pre-existing difficulties include anatomicopathological findings suggestive of chronic stress and hypoxia; abnormalities in sleep state, cardiorespiratory function, and tissue oxygen utilization; postnatal growth retardation; and the infant's temperament and behavioral patterns between birth and death. Currently suggested etiologic mechanisms revolve around interrelated infectious disease processes and developmental, pathophysiologic, metabolic, and environmental phenomena. A number of these phenomena are closely related to chronic stress and hypoxic states.

In FY 1978, the Institute initiated a Cooperative Epidemiologic Study of SIDS Risk Factors. The Study involves six data collection centers located in New York City, upstate New York, Chicago, St. Louis, northern California, and Seattle; a data coordinating center at the University of Washington in Seattle; and a pathology processing laboratory in the Medical Examiner's Office in San Francisco. The study aims to develop a risk prediction algorithm which can be applied to infants during the first week of life. Case-control comparisons

for each factor studied will determine the extent of SIDS risk associated with the factor. At least 812 cases of SIDS, defined by a common necropsy protocol, will be obtained. Each case will be matched with two controls. One control infant is matched for age only; the other control infant is matched for age, birthweight, and race (black/non-black), where possible.

Final interviews involving mothers of case and control infants and neighborhood appraisals pertaining to all study subjects have been completed. Abstraction of medical records began May, 1980. It is anticipated that abstraction of medical records for the first 200 cases and their matched controls (a total of 600 sets of abstraction forms) will be completed by the end of December, 1980. Entry of this data on to computer files should be completed by the Data Coordinating Center (DCC) by the end of November, 1980. Final cleaning and editing of all collected data has begun.

A descriptive comparison of data from interview responses of case and control mothers has been completed for the first 200 cases and their matched controls. These data are being utilized by pre-analysis subcommittees in developing hypotheses to be tested and strategies for analysis. Based on this small sample, it appears that some findings from previous studies will be confirmed and that new information about the pregnancy and neonatal histories of the mother-SIDS infant dyad will emerge.

Smoking in Pregnancy

In FY 1980 the Section continued an active participation in the Department's initiative on the Health Consequences of Smoking. Staff assumed responsibility for the organization and preparation of a manuscript updating current knowledge on the effects of maternal smoking on pregnancy and infant health. This document has been prepared as part of the 1980 Surgeon General's Report on the Health Consequences of Smoking for Women. Investigator-initiated research was stimulated by the publication in 1979 of an RFA entitled "Maternal Smoking and Pregnancy Outcome." Six grants, one Major Research Program (MRP), and one Fellowship were funded in this area for a total of $586,934.

Conferences, Staff Activities, and Publications

During FY 1980, the Section provided partial support for the Perinatal Research Society's Interdisciplinary Conference on Perinatology.

Staff members served as Institute representatives at a number of professional organizations' meetings including the Society for Gynecologic Investigation, Society for Pediatric Research, and the American Pediatric Society as well as the American Academy of Pediatrics Committee on Drugs.

Section publications include:

Hasselmeyer, E.G., Meyers, M.B., and Longo, L.D.: Pregnancy and Infant Health in The Health Consequences of Smoking for Women: A Report of the Surgeon General, U.S. Department of Health and Human Services, Public Health Service Publication No. 623-87711525, Washington, D.C., U.S. Government Printing Office 1980, pp. 224-296

Catz, C.: Congenital Malformations Registry in Prenatal Approaches to the Diagnosis of Fetal Hemoglobinopathies, edited by Y.W. Kan and C.D. Reid, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 80-1529, May, 1980, pp. 181-184.

Alexander, D.: Amniocentesis Study in Prenatal Approaches to the Diagnosis of Fetal Hemoglobinopathies, edited by Y.W. Kan and C.D. Reid, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 80-1529, May, 1980, pp. 171-179.

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Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Number: N01 HD 7-2839

Contract Title: Data Coordinating Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: University of Washington
Seattle, Washington

Money Allocated: $204,259 (January 1, 1980 to December 31, 1980)

Objectives: This contract serves as the Data Coordinating Center to the NICHD Cooperative Epidemiologic SIDS Risk Factors Study. It is responsible for the processing, management, and analyses of data collected by the participating Study Centers, N01 HD 7-2839, University of Washington, Seattle; N01 HD 7-2840, University of California, Davis; N01 HD 7-2841, Health Research, Inc., Albany, New York; N01 HD 7-2842, Medical and Health Research Association of New York City, Inc.; N01 HD 7-2843, Loyala University of Chicago; and N01 HD 7-2844, St. Louis Regional Maternal and Child Health, Inc.,

Findings: The study design is completed. Protocols, questionnaires, medical record abstraction forms and reporting forms for the Study Centers have been developed and are in use. The Data Coordinating Center has received a total of 812 interview schedules (questionnaires) for SIDS cases, and from approximately 1600 matched controls. Slide review data from the Pathology Coordinating Laboratory has been received for about 150 cases. Abstraction of death investigation reports began on August 1, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study ended December 31, 1979. Cleaning and editing of data by Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in August, 1979. A final report on approaches to data analyses will be completed by September, 1980. Study results on the first 200 cases will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2839

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: University of Washington
Seattle, Washington

Money Allocated: $63,643 (January 1, 1980 - December 31, 1980)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. More than 800 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The other five data collecting centers include: N01 HD 7-2840, University of California, Davis; N01 HD 7-2841, Health Research, Inc., Albany, New York; N01 HD 7-2842, Medical and Health Research Association of New York City; N01 HD 7-2843, Loyola University of Chicago, and N01 HD 7-2844, St. Louis Regional Maternal and Child Health, Inc. The data coordinating center is located at the University of Washington (N01 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers. Abstraction of death investigation reports will begin in late summer, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study ended December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in August, 1979. A final report on approaches to data analyses will be completed by September, 1980. Study results on the first 200 cases will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2840

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: University of California
Davis, California

Money Allocated: $101,774 (January 1, 1980 - December 31, 1980)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. More than 800 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The other five data collecting centers include: N01 HD 7-2839, University of Washington, Seattle; N01 HD 7-2841, Health Research Inc., Albany, New York; N01 HD 7-2842, Medical and Health Research Association of New York City; N01 HD 7-2843, Loyola University of Chicago; and N01 HD 7-2844, St. Louis Regional Maternal and Child Health, Inc. The data coordinating center is located at the university of Washington (N01 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers. Abstraction of death investigation reports will begin in late summer, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study ended December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in August, 1979. A final report on approaches to data analyses will be completed by September, 1980. Study results on the first 200 cases will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: NO1 HD 7-2841

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Health Research, Inc.
Albany, New York

Money Allocated: $105,736 (January 1, 1980 - December 31, 1980)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. More than 800 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The other five data collecting centers include: NO1 HD 7-2839, University of Washington, Seattle; NO1 HD 7-2840, University of California, Davis; NO1 HD 7-2842, Medical and Health Research Association of New York City; NO1 HD 7-2843, Loyola University of Chicago; and NO1 HD 7-2844, St. Louis Regional Maternal and Child Health, Inc. The data coordinating center is located at the university of Washington (NO1 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers. Abstraction of death investigation reports will begin in late summer, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study ended December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in August, 1979. A final report on approaches to data analyses will be completed by September, 1980. Study results on the first 200 cases will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2842

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Medical and Health Research Association of New York City, Inc.
New York City

Money Allocated: $104,620 (January 1, 1980 - December 31, 1980)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. More than 800 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The other five data collecting centers include: N01 HD 7-2839, University of Washington, Seattle; N01 HD 7-2840, University of California, Davis; N01 HD 7-2841, Health Research, Inc., Albany, New York; N01 HD 7-2843, Loyola University of Chicago; and N01 HD 7-2844, St. Louis Regional Maternal and Child Health, Inc. The data coordinating center is located at the university of Washington (N01 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers. Abstraction of death investigation reports will begin in late summer, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study ended December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in August, 1979. A final report on approaches to data analyses will be completed by September, 1980. Study results on the first 200 cases will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2843

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Loyola University of Chicago
Chicago, Illinois

Money Allocated: $131,865 (January 1, 1980 - December 31, 1980)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. More than 800 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The other five data collecting centers include: N01 HD 7-2839, University of Washington, Seattle; N01 HD 7-2840, University of California, Davis; N01 HD 7-2841, Health Research, Inc., Albany, New York; N01 HD 7-2842, Medical and Health Research Association of New York City; and N01 HD 7-2844, St. Louis Louis Regional Maternal and Child Health, Inc. The data coordinating center is located at the University of Washington (N01 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers. Abstraction of death investigation reports will begin in late summer, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study ended December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in August, 1979. A final report on approaches to data analyses will be completed by September, 1980. Study results on the first 200 cases will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2844

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: St. Louis Regional Maternal and Child Health, Inc.
St. Louis, Missouri

Money Allocated: $51,298 (January 1, 1980 - December 31, 1980)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. More than 800 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The other five data collecting centers include: N01 HD 7-2839, University of Washington, Seattle; N01 HD 7-2840, University of California, Davis; N01 HD 7-2841, Health Research, Inc., Albany, New York; N01 HD 7-2842, Medical and Health Research Association of New York City; and N01 HD 7-2843, Loyola University of Chicago. The data coordinating center is located at the University of Washington (N01 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers. Abstraction of death investigation reports will begin in late summer, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study ended December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in August, 1979. A final report on approaches to data analyses will be completed by September, 1980. Study results on the first 200 cases will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: NO1 HD 8-2845

Contract Title: Pathological Coordinating Laboratory (PCL) for the NICHD Cooperative Epidemiologic Study of SIDS Risk Factors

Contractor: Office of Medical Examiner
City and County of San Francisco
San Francisco, California

Money Allocated: $23,852 (September 30, 1979 through September 29, 1980)

Objectives: This contract serves as the Pathology Coordinating Laboratory to the NICHD Cooperative Epidemiologic SIDS Risk Factors Study. The Laboratory provides essential services to ensure the accurate diagnosis of SIDS. The necropsy, a common gross dissection protocol, will be followed to dichotomize the dead infants into "definitely not SIDS" and "eligible for further study". The contractor will process tissues submitted by six Study Centers and will produce a set of stained slides for each infant entered into the study.

The six data collection centers are: NO1 HD 7-2839, University of Washington, Seattle; NO1 HD 7-2840 University of California, Davis; NO1 HD 7-2841, Health Research, Inc., Albany, New York; NO1 HD 7-2842, Medical and Health Research Association of New York City; NO1 HD 7-2843, Loyola University of Chicago; and NO1 HD 7-2844, St. Louis Regional Maternal and Child Health, Inc. The data coordinating center is located a the University of Washington (NO1 HD 7-2839).

Findings: A common gross dissection protocol has been developed. Medical Examiners serving the six Study Centers have been following the protocol since the start of data collection (October 9, 1978). As of June 1, 1980, the PCL has received tissues from 981 infants who have died. Of these deaths, more than 800 are believed to be true SIDS cases, the balance includes quality control cases and some cases where subsequent diagnosis elicited a cause of death. It is expected that the review of all slides by the Pathology Study Panel will be completed by December 31, 1980. Abstraction of Death Investigation Reports and Autopsy Reports should be completed early in 1981.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Pregnancy and Perinatology Section
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

ontract Number: N01 HD 9-2828

Contract Title: Non-Human Primate Colony

Contractor: University of California, Davis, California

Money Allocated: $194,142 (October 1, 1979 to September 30, 1980

Objectives: Under this contract, a colony of Rhesus monkeys (Macaca mulatta) of known medical, reproductive and genealogical history is being kept and developed at the California Primate Research Center, University of California at Davis. This colony serves as a resource of research animals of known quality from a standardized environment for investigators whose research is funded by the NICHD.

Procedures: Animals of different ages including dated pregnancies, embryonic and fetal material, neonates of known gestation, mother-infant pairs, juveniles of known age, as well as certain biopsy material, tissues, and fluids are being made available to selected investigators, all of whom must be directly supported by NICHD. Application for animals is competitive and allocation is determined by an advisory committee which also advises on colony and contract management. Animals are shipped to recipient scientists throughout the country; a limited number of investigators can also be accommodated to visit facilities on-site. Biological and behavioral data profiles are compiled and incorporated into the computerized record of each animal. This information is available to recipient investigators.

Significance: Demand for animals from this resource reflects the use of Rhesus monkeys as a model for the human in biomedical research.

Proposed Course: The size of the colony, stabilized at 335 animals, is adequate for the projected demand of 80-100 pregnancies per year plus the need of replacement breeder stock.

Project Officer: Charlotte Catz, M.D.

NICHD Annual Report
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Contract and Collaborative Research

Contract Number: N01 HD 5-2855

Contract Title: Evaluation and Follow-up of Selected Respiratory, Cardiac and Neurophysiologic Parameters in Infants as Indicators of Risk for the Sudden Infant Death Sydnrome (SIDS)

Contractor: Montefiore Hospital and Medical Center, Bronx, New York

Money Allocated: $8,600 (September 30, 1979 - March 29, 1980)

Objectives: The objectives of this project were to study cardiorespiratory-neurophysiologic phenomena which may be relevant to the understanding of sudden infant death syndrome (SIDS) and to identifying infants at risk.

Findings: Complete long-term recordings were obtained on 31 near-miss infants and 15 control babies. The contractor has delivered to the Institute a clean and edited tape of all data obtained with appropriate documentation. The development of a home monitoring equipment was advanced and a prototype is available for further field testing. New methodologies were examined in order to permit the direct reduction and analysis of data stored on cassette tapes used in home environments.

Proposed Course: The contract terminated May 31, 1980.

Project Officer: Charlotte Catz, M.D.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Pregnancy and Perinatology Section
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Number: N01 HD 5-2856

Contract Title: Evaluation and Follow-up of Selected Respiratory, Cardiac and Neurophysiologic Parameters in Infants as Indicators of Risk for the Sudden Infant Death Syndrome (SIDS)

Contractor: Columbia-Presbyterian Medical Center, New York, New York

Money Allocated: $294,979 (July 1, 1980 - June 30, 1981)

Objectives: The objectives of this project are to study cardiorespiratory-neurophysiologic phenomena which may be relevant to understanding the sudden infant death syndrome (SIDS) and identifying infants at risk.

Findings: Healthy infants and infants with "near-miss" episodes are monitored during sleep in a non-invasive manner. Data obtained include recordings of EEG, EOG, EMG, EKG, respiratory rate and respiratory volume. Data collection has been completed on 28 near-miss infants, and 21 normal babies. New statistical methods have been developed to determine the interrelationships between cardiac and respiratory parameters. It has been shown that breathing during sleep is cyclic, possibly related to neural mechanisms but not dependent on sleep state. Furthermore, it appears that inspiratory time and expiratory time of a single breath may be controlled by different mechanisms. Findings related to QT intervals in the EKG are being re-analyzed taking into account heart rate, which is faster in the near-miss group.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between the age of one month to one year. This proposal is developing new information that will help to identify infants at risk for SIDS. This study is an integral part of the Institute's SIDS research contract program to develop further knowledge on the causes and underlying mechanisms of SIDS.

Proposed Course: A renewal proposal is approved for a final period of 18 months.

Project Office: Charlotte Catz, M.D.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Human Learning and Behavior Branch

The Human Learning and Behavior Branch has a primary interest in and responsibility for studies of normal behavioral, psychological, social and psychobiological development, from infancy through childhood and adolescence up to and including early naturity. Its program responsibilities also include studies of those prenatal and postnatal processes and factors, within and without the individual, that result in developmental delays or defective development, leading to specific dysfunctions, defects or disorders of behavior in childhood and adolescence (excluding disorders classified as, or closely related to, mental retardation and to mental illness and emotional disorders).

The program has been divided into five major areas: (1) developmental behavioral biology, or psychobiology; (2) cognitive development, including learning, perception, thought, memory; (3) development of communicative abilities, including speech, language, and reading; (4) social and affective development; (5) illness-related and health-related behaviors, including behavioral medicine and environmental factors affecting behavioral development. Processes and factors specific to each stage of development are studied, including infancy, early childhood, preadolescence, adolescence and young adulthood. Longitudinal and life-span behavioral studies, including developmental antecedents of certain disease states in adults and behavioral development of children born at biological risk, are also included.

BIOLOGICAL BASES OF BEHAVIORAL DEVELOPMENT

Developmental Behavioral Genetics

A small but active program of research in behavioral genetics is supported by the Human Learning and Behavior Branch. It consists of two types of research - experimental, animal studies and human studies, each differing from the other in a number of ways, methodological, conceptual, and phenomenological. One such animal study, for instance, is studying the interaction of genetic, maturational, and experimental variables in the phenotypic expression of a very specific form of behavior. Essentially, a population approach to the study of individual differences of behavior is being used, following the emergence of specific color-preference behavior in a population of pure-bred quails, through some generations, systematically varying the early experience of the quail (exposure to red or blue or white light, for varying periods of time), and studying the resultant behaviors of subsequent generations. If successful, the investigator will have established a useful model for the study of the interaction between specific, genetically based behavior and experience.

Another Institute-supported study is engaged in determining the relative influence of genetic and environmental factors in visual perception, in human beings. Four hundred families are under study, 200 European, 200 Japanese, each family consisting of two parents plus two older children.

Some 25 separate visual measures will be taken from each subject, such as visual acuity, brightness and line sensitivity, stereopsis, and eye dominance. Since it is known that racial differences of a genetic nature exist in sensory processing characteristics, it is possible that single-gene effects will be found. If so, likange with various blood group and protein markers, already obtained from these subjects, will be examined, as well as associations, with age, sex, socioeconomic status, and previously collected data on cognitive performance.

Developmental Neurophysiology and Behavior

Brain Laterality. In recent years there has been a growth of interest in the development of lateralization of brain function during childhood, an area hardly studied previously. Whether lateralization is complete very early in life, possibly even at birth, or is not complete until late childhood is at the present time an open question. To date there have been relatively few electrophysiological studies of lateralization of function in infants, preschoolers or young school-age children, even though it is precisely at these times that such right-left specialization is occurring. It is particularly important to pursue such developmental lines of inquiry if we are to address successfully the problems raised by language and learning-disabled children, for assuredly what we have learned from adult studies will not be sufficient to answer the questions posed by the broad range of developmental disabilities now occurring. With regard to lateralization of language function, it is quite possible that different levels of language processing are lateralized to a different degree at any one time, and develop at different rates. Undoubtedly, there are differences of timing in the development of the ability to receive language, and to produce language, for instance. Happily attention is now turning to these and other related questions of a developmental nature, with several important investigations currently under NICHD grant support. One such study, for instance, is engaged in assessing hemispheral specialization for speech processing during infancy and early childhood. Auditory evoked brain potentials will be recorded from the left and right temporal regions, using both verbal material and musical passages, in children from 2 months to 7 years of age, to determine whether and to what extent hemispheric specialization occurs during this age period.

Neurophysiological Basis of Cognition. There is now occurring a general shift of interest toward understanding the neurophysiological basis of cognitive processes: thought, perception, memory, and attention, as well as language and reading. In part this is because newer and better methodologies are being developed, including the contributions of computerized technology; in larger part because of the increased attention and concern over the apparent increase in the incidence of learning, speech,and reading problems. One such study is investigating the ontogeny of the late-occurring EEG wave in children, utilizing visual and auditory stimuli which are sufficiently interesting or novel to elicit complex cognitive activity. Normal children from ages one through ten are being studied, as well as children with learning disabilities, cognitive problems, dyslexia, etc. The results of this study will not only be of theoretical interest but will have practical implications as well, for if successful it should produce an objective test of the adequacy of cognitive functions in infants and children.

Neuroendocrines and Behavior

Since the 1950's, it has been known that sex hormones have a direct influence on the developing brain, resulting in characteristic male or female behavior. It seems, in fact, that mammalian behavior patterns are basically female and that male patterns are induced by the action of the sex hormone testosterone on the brain of the animals, either before or after birth. A natural extension of this finding is to determine whether there are other resultant behavioral differences between male and female. Some initial explorations in this area (dealt with elswhere in this report) suggest that there may well be such differences - in temperament, perceptual capacity, cognitive style, affectional expression, etc. The questions to be answered are not simply whether, but when, how, to what extent, and under what varying circumstances, experiential or otherwise, such differences occur.

It is also important to ask whether other hormones of the endocrine system, for example, the thyroid or adreno-cortical hormones, also have an influence on the developing brain. If so, what is the nature of that influence, at what stages of development does it occur, and what specific brain structures are affected?

In addition to research on the relationship between hormones and gender-specific behaviors, much research has also concerned itself with the role of the pituitary-adrenal system in modulating behavioral responses. As a result, in part, of Institute-supported animal research, it is well established that this system is part of an arousal system, sensitive to stress.

Further studies, now in progress, are concerned with the role of the neuroendocrine system in the social bonding, or attachment, of the mother to the infant, and the infant to the mother. Until recently, most studies have been concerned with the effect of stress, such as separation, on the infant, with little or no attention being paid to the neuroendocrine responses occurring in the mother. This particular series of studies will continue to examine the effects of separation on both mother and infant, the specificity of response, and the role of environmental factors.

It is known in general that stressful experiences during early development, if sufficiently severe and prolonged, can have permanent consequences. How severe, for what periods of time, at what stages of development such stresses must occur to result in severe maladaptive behaviors in later life, and what the neuroendocrine and other physiological concomitants and sequelae of such events are, represent questions now under study by a number of Institute supported investigators. Finally, answers are sought to the questions of whether, how, and to what extent such maladaptive behaviors can be changed, and what role behavioral and social variables play in such a process.

Much experimental work has been done in animals on the role of the endocrines in sexual development, including central nervous system-hormonal interactions, and in modulating responses to stress or arousal. Likewise, there has been much clinical work in humans during infancy, childhood, and adolescence, on such conditions as hypothyroidism, abnormalities of sexual development, and disorders of pubertal onset. However, although some research has been done

which relates normal somatic and physiological growth, as modulated by the endocrine system, to normal psychological and behavioral development in humans, much more needs to be done.

The Branch plans to stimulate and support such research, particularly during infancy and early childhood and during adolescence.

LEARNING AND COGNITIVE DEVELOPMENT

Cognitive Development in Infancy

Almost half of the NICHD grantees who are studying perceptual, cognitive, or social development during childhood, are working with infants. In fact, the most intensive research focus has been on the first year, and often the first few months of life. NICHD involvement has paralled and contributed to the very rapid growth of this field.

The roots of all mental activity and social behavior may be traced to early infancy. However, until fairly recently the infant was considered a rather passive, incompetent being with little power to perceive, comprehend, or influence his or her environment. Recent methodological advances have made it possible to measure the responses of very young infants to a variety of physical and social stimuli, thus providing valuable new information about infants' perception, learning, and memory. The rapid changes which take place in these processes have also been traced.

Research of this sort has attracted a vigorous group of researchers supported by the Institute. Our grantees have used overt motor responses, such as eye and head movements and sucking, as well as covert physiological responses such as heart rate, to measure acuity, attention, learning, and memory for tones, brightness, and hue, and also for visual and auditory patterns. These researchers have determined which stimuli can be discriminated, which are given attention and preferred, and which are recognized and remembered. With these methods researchers have traced the behavioral concomitants of the early maturation of the sensory and nervous systems and the growth of learning ability and cognitive capacities. It therefore becomes possible to more accurately assess the condition of infants who are born at risk, and the effects of environmental regimes, programs of stimulation, parent education, and the like.

The infant's visual system and responses to visually presented stimuli have been most intensively studied. The visual system is immature at birth but matures rapidly in the first few months of life. The Human Learning and Behavior Branch has supported several researchers who have studied the increases in acuity which are typical during these months. Astigmatism and strabismus has also been studied, and the advances which have been made provide the basis for earlier and more accurate diagnosis than has been possible hitherto.

Although the visual system has been most thoroughly researched, in the last few years the auditory system has also been the focus of much attention. However, only a few NICHD researchers have investigated auditory perception in early infancy. Those who have done so have studied infants' responses to speech and nonspeech sounds, and have found that young infants are surprisingly

able to discriminate certain speech sounds.

The greatest emphasis has been placed, not on what can be seen and heard, but on how the infant perceives the world about him, that is, visual forms and objects, and visual and auditory patterns in pictures and in speech, and how the infant integrates information which comes from different sensory modes. The Branch has supported developmental studies of depth perception in infants, auditory-visual relationships, auditory localization, and visual and tactile exploration of objects which the infant may manipulate. For example, one researcher is currently studying the range of behaviors and the extent of stability which may be expected in individual infants' exploration of objects, and comparing these behaviors to the behaviors of low-birth-weight babies.

Increased knowledge about sensory and perceptual abilities makes possible increased understanding of infants' capacities to learn, understand, and remember his world. The study of habituation, that is, a decreased response to repeated presentations of a stimulus, allows inferences to be made about what stimuli can be discriminated from each other, recognized and remembered. NICHD researchers have studied recognition of objects and persons, memory for briefly presented stimuli, and stimuli presented in various contexts. Although it had once been thought that the infants' world was a "buzzing, booming," world of unrelated sensations, it now appears that even newborns can perceive far more about their surroundings than was previously thought to be the case. The rapid changes in visual and auditory perception which take place in the earliest months of life have made early infancy a particularly fertile period for study. The results of this research have added to our basic theoretical knowledge about native human abilities and their interaction with experience. It has also been a fruitful source of practical information which is useful in the assessment and treatment of infants and young children.

Cognitive Development in Childhood

Despite the concentration of research effort upon early infancy, many grantees have been interested in those changes in perceptual, learning, cognitive, and memory processes which begin the child's second year of life, accompanying the emergence of language and continuing throughout childhood. Perception and discrimination learning is far more efficient when the child is able to focus attention on relevant features of stimuli and, thus, researchers have concentrated upon age changes in the child's ability to distribute attention effectively, and on those aspects of stimuli which are distinctive, or salient to the child. Children appear to proceed through several age-related stages during which particular characteristics of stimuli, e.g., color, become especially salient. Later, children are able to attend to, and keep in mind more than one dimension, e.g., color and form, when discriminating between stimuli. Often children have particular difficulty discriminating and representing the orientation of two-dimensional forms, leading, for example, to early problems in writing and sometimes in reading. Therefore researchers have emphasized perception and memory. Several grantees have traced the course of acquisition of the ability to discriminate orientation in an orderly fashion, first discriminating upright from nonupright, inverted from sideways, and finally, right from left.

Other researchers have been studying the development of the perception and recognition of faces, voices, and gait. These aspects of person recognition are especially useful in cross-cultural studies of child development, since they are universally important, and are not usually taught in school. There are very rapid increases between age six and ten in the development of the ability to recognize faces. One NICHD grantee is gathering normative data about the development of facial perception and relating it to the rate and status of maturation of the right hemisphere of the brain and exploring the possible diagnostic use of tests of this type. Another grantee studying facial perception has been emphasizing the changes in children's strategies for memory for faces which occur with age.

The recent resurgence of psycholinguistic research has led to greater emphasis upon auditory perception of speech and nonspeech sounds in infancy and the development of the child's ability to discriminate auditory stimuli and patterns. Although visual perception has traditionally received far more attention than auditory perception and continues to be a major research focus, in the last few years NICHD grantees have developed techniques for studying perception, processing, and memory for auditory stimuli, particularly speech sounds. NICHD grantees are studying the perception of intensity (loudness), frequency (pitch), and the ability to localize sound sources. The ability to recognize or recall musical patterns is also being studied. This work forms an important base for the study of speech production and perception, and of the role of auditory feedback on speech development and production.

Several grantees have actively researched the effects of age and instruction upon preschool and school age children's ability to learn and remember linguistic and pictorial types of material. Deficiencies in the child's labeling, classification, and organization of material results in learning difficulties which are characteristic of young children and some learning-disabled children. An important source of difficulty for memorization has been found to be the lack of an effective rehearsal strategy. Children who have developed such strategies for remembering are better able to withstand the effects of time and distraction upon learned material than children without such strategies. They are often able to use devices intended to assist memory (mnemonics), such as the proverbial string around the finger, or the use of a rhyme.

A related field, currently receiving considerable attention, is concerned with children's awareness of how one learns and remembers, that is, metacognition and metamemory and the contribution of such knowledge to the processes of learning and memory. In contrast with adults, children seem less likely to monitor their own understanding and memory, to try to find out whether they understand something and will remember material in the future.

The research funded by NICHD, thus, typifies the trend toward the study of the child as an active organizer of information about his environment. The results of this research make possible new knowledge about normal developmental changes and the processes by which children become more effective at mastering their environment. This knowledge has obvious value in understanding developmental delays, learning disabilities, and mental retardation. It does appear that many children who have been labeled as "learning disabled" have particular problems organizing information that is to be remembered. Often, instruction

or the provision of memory aids results in a narrowing of the gap between the performance of normal and learning-disabled children.

The study of the child as an active organizer of his environment is also far more useful in understanding the child's gradual acquisition of complex concepts than the heretofore popular associationist view that learning represents the establishment of a connection or bond between stimuli responses. The emphasis upon how the child structures his own knowledge of the world has led to productive research, for example, on how the child forms mental constructs of the three dimensional spatial world and how he uses this information to remember his immediate and more distal environment. New emphasis is being placed upon the child's comprehension of material and the role of meaningfulness in memory. Older children make more inferences about causality in a chain of events, connecting or filling in gaps in narrative material, for example. Memory for narratives is thus aided. Once the content of a narrative is comprehended, as a chain of causal events, the child uses this representation of the story content to retell the narrative, answer questions, draw further inferences, and to summarize the story. Similar studies are also being undertaken by several of our grantees on the development of comprehension of material presented in the visual mode, such as two-dimensional visual patterns and spatial layouts.

HUMAN COMMUNICATIVE PROCESSES

Speech and Language

Speech is important in human development and in behavior. People like to talk, and speech is an efficient method of communicating as compared to all other modes of communication. Speech serves both as a signal, shaped by its method of production, and as a signal, tailored for perception. It serves as a carrier, first, mainly, of phonetic messages, then as a carrier of cues to all levels of language. Although we have left behind the idea of speech "merely as sound stuff, or even spectrum stuff that has certain characteristic frequencies" (Frank Cooper, Haskins Laboratories), investigators are still addressing themselves to fundamental questions regarding the nature of speech and language: How is it that a series of sounds, meaningless in themselves, come to have the capacity of transmitting information from one person to another? How does this process occur? How does a language, with its complex set of rules, evolve from such an innate capacity? How does the individual infant or child learn this strange code? What in fact are the rules whereby information is extracted from sound itself and transmitted to others as information? How does it develop within each single individual, and what are the physiological and psychological factors which make up this system?

The Human Learning and Behavior Branch has provided continuing research support to help answer these and related questions. For example, the Branch has supported studies of how young children make auditory distinctions that correspond to adult phonological boundaries. This research has revealed that even very young infants can discriminate between speech phonemes (sounds) in the very same manner as adults. Other studies suggest that, well before the infant begins to use speech sounds, he has been "programmed" to produce certain sounds, and not others, by the characteristics and limitations of the

speech of those who brought him up. It has also been shown how perception in infants becomes restricted by the culture in which the infant is brought up - by the phonetic boundaries of the language it is trained to use. Japanese infants can easily distinguish the "r" and "l" phonemes, whereas Japanese adults are unable to make such distinctions.

Children with hearing handicaps are known to have difficulty acquiring normal speech and language, yet many of the underlying relationships between normal and partial hearing, and the acquisition of communicative skills remain unanswered. In order to elucidate some of these unknowns, the HLB Branch supports research projects concerning the child's ability to respond to acoustic stimuli both for pure tone and for language-related complex sound. One investigator, for example, has demonstrated that the heart rate and brain waves of newborns can be differentially changed by pure tone and complex speech-like acoustic signals. When fully developed into a clinically applicable technique, this procedure should prove a reliable and valid measure of an infant's ability to hear speech.

Sign Language

A very interesting development of recent years has been the serious scientific study of a language long in use but generally dismissed as not a true language but rather a crude system of communicative gestures, namely, American Sign Language (ASL). This is the system of hand signs that has developed among deaf people in the absence of speech. Study of this system shows that it has developed into a true, autonomous language, with its own inner form, using a visual-manual system of communication rather than the auditory-vocal system of language native to man. Not only does ASL have morphemes (visual signs) comparable to those used in speech (words, syllables and letters), but it has a structured method whereby these lexical units are modified, embedding the sign form within geometric patterns, such as circles, lines, and arcs, and imposing dynamic changes of quality on the sign, such as length, contouring, acceleration, and the patterned alternative use of the two hands, comparable to the use of pitch, stress, articulation, etc., in speech.

These findings suggest that the human capacity for constructing and utilizing a complex language system is independent of the specific sound modality in which language originated. Until recently, all information about human lan-- guage processing has come from the study of spoken language. It has now become possible to determine what aspects of the overall form of language are determined by the specific method of production and reception used, and what aspects of its form and organization are determined by more purely cognitive and perceptual capacities and processes. It is now possible, for instance, to determine the psychological processes utilized in the perception and memory of a language in the manual-visual mode, as well as in the vocal-auditory mode, and to contrast the two. Such studies are now being carried out by Institute-supported investigators.

A particularly intriguing aspect of these studies is the opportunity to test current theories of cerebral dominance, or brain lateralization, since analytical, sequential processes, such as language, are primarily the function of the left hemisphere, whereas spatial processes, such as sequences of skilled

motor movements, are commonly assigned to the right hemisphere. Whether lateralization of ASL turns out to be dominated by either the left or the right hemisphere, the information obtained will help to clarify the relative importance of language versus visual-spacial processing in determining hemispheric asymmetry.

Language in Animals

Several investigators in recent years have attempted to demonstrate that animals, at least the higher apes, have the capacity to learn and use language, under human tutelage. The basic questions being addressed are whether language is indeed unique to the human species, as long believed, or exists in other animal species as well, and, if the latter, what is the nature of the interrelationships, in animals and humans, between communication, cognitive processes, or intelligence, and social interaction. Despite impressive evidence of the ability of chimpanzees to use simple signs in combination to communicate meaning, it remains to be shown that they can manipulate and order such signs in the manner characteristic of human grammatical language, and that they are engaged in anything other than a complex form of operant behavior.

Whatever the final scientific outcome of these intriguing and controversial studies, such efforts are serving to demonstrate, on the one hand, that animals have greater cognitive capacities than previously recognized i.e., that animals are more clever than (some scientists) thought, and, on the other hand, that the uniqueness of human language lies not simply in the ability to use symbols to convey meaning, but in the manner and extent to which signs are ordered and processed to permit the production of an infinite number of messages at will. (After all, the lion's roar, meaningless in itself, conveys meaning to others, as a threat of things to come.) An interesting and important outcome of one of these projects, currently receiving Institute support, is the successful application to rather severely retarded humans of the techniques used to train chimps to communicate with their trainers, such that some of these previously non-verbal patients have begun to use speech for the first time.

Reading and Dyslexia

Since 1964, when the first NICHD Advisory Council recommended that the Institute concern itself with the large number of children who do not learn to read, the problem of specific reading disability, or dyslexia has been a continuing priority area of research for the Institute. Dyslexia is a puzzling and complex disorder, varying from a relatively pure form of reading disability to cases in which many other symptoms occur, such as disorders of memory, attention and comprehension, plus hyperactivity, and lack of motor coordination at times. Although a neurophysiological basis for dyslexia has not been proven, differences between normal and dyslexic children have long been shown to occur in the recordings of brain electrical activity, including lateralization of brain function. No consistent pattern of such responses, however, has yet been found. Finally, in about one third of all cases, there is strong evidence of a familial tendency in dyslexia, possibly genetic in origin.

One important question repeatedly asked is whether reading and listening can

be regarded simply as parallel processes occurring in different modalities, converging at some point on a common linguistic path, or whether there are significant differences between the two processes. Although some children may pick up the written system on their own, the vast majority acquire it only after exposure to a formal teaching procedure. In contrast, with the exception of children with severe handicaps, such as deafnesss or brain damage, all achieve skill with oral language. Evidently these two cognitive processes, speech and reading, though closely related, are different in some very crucial ways. Institute-supported research shows that written language, far more than speech, places a direct demand on the individual's acquired knowledge of language - what has been called grammatical knowledge; and that such knowledge, consequently, must be accessible to the reader, as it is to the language learner. Although reading and listening use the same analytic mechanisms, a phonetic recording analysis of a sentence is accomplished in reading, unlike listening, by means of a language-acquisition procedure. Thus, reading has much in common with language acquisition, and the child who has learned to use and understand language beyond what is required for simple speech is more likely to learn to read easily than the child whose language acquisition capacity has not progressed in such a manner.

Neuropsychology of Dyslexia. An on-going study has recently found that neurophysiological aberrations in the brains of dyslexics are more widespread than previously suspected. Mapping of brain electrical acitvity found four distinct areas of the brain in which the electrical recordings from dyslexics differed from normals, involving both hemispheres, and occurring both at rest and under activated testing conditions. One cannot yet say whether such data have sufficient specificity to permit clinical differentiation of dyslexia from other learning disabilities. It does suggest, however, that such neurophysiological testing may someday prove as useful in distinguishing various forms of learning disability as is now true for the epilepsies.

Genetics of Dyslexia. A human genetic study currently under way is attempting to identify several etiologically distinct subtypes of reading disability. Although, as previously stated, there is evidence of a familial tendency in reading disability, the population as a whole appears to be genetically heterogeneous, leading investigators to attempt to determine whether there exists a specific subgroup of dyslexia which exhibits true dominant inheritance. The research is being limited to a study of chromosome 15, which has previously been identified as linked to the occurrence of dyslexia, and will utilize genetic linkage and localization techniques. It is hoped that this study will help to determine more clearly than have studies before this, whether or not reading disability is a unitary, homogeneous sttribute; whether the familial component known to exist among dyslexics is truly genetic in nature, at least for s subset of dyslexics; and, if a positive finding of a dominant gene linked to chromosome 15 should result, whether the overt, (phenotypic) expression of the genotype is relatively uniform or relatively variable. If genetically distinct sub-types of dyslexia should be found, a major step toward clarification of this confusing field will have been taken, comparable to the discovery of trisomy in Down's syndrome in the field of mental retardation.

SOCIAL AND AFFECTIVE DEVELOPMENT

The Human Learning and Behavior Branch program in young children's social and affective development is small in comparison with the other programs of the Branch. However, this program sponsors a dozen or so highly competent researchers who are engaged in a variety of interesting and productive projects. Although some of these researchers have studied either infants or older children, approximately half have studied a broader age range from below age two through the late preschool or early grade school years. In several cases the same group of children has been followed in short-term longitudinal, or follow-up studies. The diversity of content encompassed by these investigations is representative of the present state of the field, which is currently enjoying very rapid growth.

There is a marked interest in factors which contribute to social development such as physiological and cognitive processes taking place within the child, the child's social environment, including parents and peers, and often the interactions between several of these factors. One large project is tracing the development of several cohorts of children and studying the association between levels of sex hormones in blood taken from the cord at birth, with preschool boys' and girls' temperament and typical gender-related behavior, such as rough and tumble play. An interesting and unexpected finding of this research is that birth order and spacing between births affected hormone levels as well as behavior.

The recent growth of knowledge about cognition has also led to a group of important investigations of social cognition. These studies trace the growth of children's ability to understand the emotions, motivations, and goals of others, the ability to infer the causes and outcomes of social acts and to make attributions about other individuals' personality characteristics.

Researchers in the field have also moved from a preoccupation with children's aggression to a newer emphasis upon prosocial behavior, such as generosity, sharing, helping, and caretaking. One researcher who is engaged in longitudinal and cross-sectional studies of this type of behavior in one- to three-year-olds, is constructing a scale of social development. She notes that no current theory predicts a "prosocial infant." Nevertheless, these behaviors are common in the very young. Another grantee is studying children's beliefs about the desirability of generosity and helping behaviors, contrasted with actual behaviors, and relating both to parents' techniques for motivating children to help and to share.

Still another focus of recent research is the study of relationships between peers and the growth of social effectiveness in the context of children's friendships. Same-age and cross-age interactions between young children have also been intensively studied and may offer valuable data which will aid in the treatment of "isolate" behavior in children. Again, studies by several grantees include the diverse ways in which cognitive variables contribute to social development. For example, studies of peer behavior includes a focus on problem solving within children's groups and on children's use of rules.

There has been considerable interest in the stability of individual differences in personality and social behavior. Several researchers have investigated individual differences in children's curiosity and exploratory behavior and the success of their peer interactions. A high degree of stability has been found for these individual differences, even from the first few years of life. One grantee has related the quality of the infants attachment to its mother to these characteristics. In fact, the security of the infant's attachment to its major caretaker was reliably related to the child's problem-solving competence as late as age four.

Although the Human Learning and Behavior Branch program of research in social and affective development is an excellent one, it is in need of expansion. There is, in particular, a need for research directed to children's behavior in natural settings, especially with parents and siblings within the context of the various family forms which are common today. The complex sources of the development of sex differences and gender roles are in need of study. Research on development in the middle years of children, and studies of children's social development should be moved beyond their present largely cognitive focus to include an emphasis on affect and motivation, as well as on the specific social contexts and role relationships in and outside of the family which serve to help a child define his or her own sense of being and sense of relatedness to others.

HEALTH BEHAVIOR

As a result of a 1979 initiative on "health promotion and the prevention of smoking and other behaviors detrimental to health," the HLB Branch has noticeably strengthened its support of research on children's health behavior and illness. The increased interest in this research area is part of a larger research trend in behavioral medicine, a new interdisciplinary field concerned with psychosocial and cultural influences affecting physical health and disease. The Branch particularly encourages research on such broad issues as:

(a) The behavioral factors associated in children with physical diseases and illness, acute and chronic. Relevant research encompasses the psychosocial adaptation of the child and its family to being ill, hospitalized, or in pain, including such chronic diseases as diabetes, kidney disease, and epilepsy; also the role of stress factors in disease and illness.

(b) The impact of environmental conditions on children's behavior, e.g., noise, toxic substances at home or in the atmosphere, and the effect of the habitat on the child.

(c) Health-related behaviors, specifically the development of healthy behavior in children and their families, and the prevention of such harmful health behaviors as over-eating, accident-related behavior, and smoking.

In an attempt to understand how healthy behavior develops in children and their families, two recently funded investigators are studying the development of healthy attitudes and practices in nursery school children in relation to personal cleanliness, nutrition, dental care, and safety. The educational

curriculum utilizes videotapes, puppets, records, role playing and field trips, plus an accompanying program for the parents.

Smoking. The most heavily supported aspect of research on children's health behavior concerns cigarette smoking in children and adolescents, reflecting the seriousness of this health problem today. In spite of the decrease in adult smoking over the last ten years, there has been an alarming increase in cigarette smoking among teenagers, principally adolescent girls. Evidence to date suggests that customary health messages appropriate to adults, with emphasis on the long-term consequences of smoking, fail to thwart teen smoking even though most children by the seventh grade acknowledge that smoking is dangerous to health. It appears that children do not respond to messages which are too generalized and future oriented (people who smoke don't all get heart attacks or die immediately); rather, messages which demonstrate the immediate physiological effects of tobacco on the subject's own body seem to have more impact on adolescents, e.g., actual tests demonstrating the young smoker's reduced lung capacity.

At a more basic level, behavioral scientists are trying to determine the major factors which influence children to initially take up cigarette smoking. Research findings implicate three sources of influence: the parents, the peers, and the mass media. Some studies have suggested that the smoking parent serves as a model for the child, based on evidence that a child is more likely to smoke if both parents smoke than if only one parent smokes or if neither parent smokes. Moreover, if an older sibling and both parents smoke, the child is about four times more likely to smoke than if no one in the family smokes. Also, if the child's best friend smokes, there is greater likelihood of the child smoking.

While adolescents acknowledge that peer pressure is one of the strongest influences to smoke, there is insufficient understanding of how such pressure operates or how it can be reduced. Even less well understood is the impact of mass media on the initiation of smoking, perhaps because it is difficult to isolate childrens' exposure to mass media from other environmental influences.

Although a number of research studies exist on children's smoking behavior, findings are not generalizable, because:

(1) results, often based on surveys, tend to focus on a specific group of children (generally middle-class whites) at one point in time;

(2) consensus is lacking on definitions of levels of smoking (non-smoker, the beginning, experiental, occasional, regular, and ex-smoker);

(3) information is almost always derived from self-reports (notoriously unreliable among children and adolescents); and

(4) control groups are seldom used. Equally limited are prevention programs developed to reduce the onset of smoking in children; they fail to utilize the existing knowledge of children's smoking behavior, and the programs are not adequately evaluated.

Thus, the alarming increase in teen-age smoking and insufficient understanding of the reasons for it or how to prevent it were the major reasons which led to an NICHD initiative, begun in 1979 to encourage behavioral scientists to study smoking behavior and the promotion of healthy behavior in children, with special emphasis on:

(1) onset of smoking behavior in childhood and adolescence,

(2) transition from occasional (experimental) smoking to habitual (regular) smoking, and

(3) research on intervention/educational programs to discourage the initiation of smoking in children and adolescents.

To date, the HLB Branch is supporting 16 researchers in the above areas. Most of them are investigating the factors responsible for the onset of smoking, and almost all are school-based studies for ease of administering questionnaires, conducting biological tests (carbon monoxide and saliva thiocyanate) to validate self-reports, and introducing intervention strategies with treatment vs. control groups. Only one study is sampling children in lower income black and Chicano neighborhoods, in addition to white middle class children. (The 1980 Surgeon General Report on Smoking and Health has urged more research on the smoking behavior of those lower class children who are not ever enrolled in school and tend to be transient; and the report also calls for further studies of cohort changes which would reflect changing adolescent social values toward smoking.) Another NICHD-sponsored investigator is attempting to identify the critical factors responsible for adolescents moving from the level of occasional (experimental) smoking to that of habitual smoking. Two grantees are studying the impact of maternal smoking in pregnancy on the subsequent behavioral and cognitive development of the child, up to 10 years of age in one study.

Following an NICHD-sponsored workshop (October 1979) on smoking behavior in children and adolescents, NICHD-supported investigators are now seeking common methodological strategies, including consensus on definitions of levels of smoking, a common core of questionnaire items, and use of specific biological tests. This collaborative effort will hopefully provide a sound research rationale and intervention strategy to deter the onset of smoking in children and adolescents.

RESEARCH TRAINING

The Human Learning and Behavior Branch has a very modest research training program, providing two types of support: Individual postdoctoral fellowships and institutional research training grants, including stipends for pre- and postdoctoral students. At the present time, the Branch is supporting 5 training grants, awarded to institutions of higher learning, including support for 40 predoctoral and 10 postdoctoral students. Most are for support of generic developmental psychology, with special emphasis in such areas as learning, human communication, and socialization.

A total of 5 postdoctoral fellows are currently receiving support for research training on such topics as the dependability of newborn behavior, visual

assymetry from infancy to adulthood, sensori-motor development in infancy, speech perception in early infancy, and speech perception in language-delayed children.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD-1-2420

Contract Title: Research Materials and Assistance for Studies of Language Development in Children

Contractor: Haskins Laboratories, New Haven, Connecticut
Alvin M. Liberman, Ph.D.

Money Allocated: $129,100

The purpose of this contract is to continue to provide expert assistance and specialized facilities to research scientists at other institutions who are engaged in studies relevant to an understanding of the development of spoken language and reading in children.

In general, three kinds of assistance have been provided to researchers working on problems related to the acquisition of speech and reading.

1. Research Materials. Stimulus tapes for a wide variety of experiments continue to be the primary assistance that the Laboratories have provided. Several methods of generating recordings suitable for research on speech are available. The techniques can be grouped either by type of stimulus (natural speech, synthetic speech, or nonspeech tones and noises) or by type of recording (single track or dual track). All combinations of these tapes have been used for one research project or another.

2. Specialized Assistance. The professional and technical staff of Haskins Laboratories makes its knowledge and skill available to the user groups. At the very least this involves having someone from the technical staff teach the user group how to operate the computer. But it often involves, beyond that, getting various kinds of help and advice about the more scientific aspects of the research from members of the Laboratories' professional staff. In the end, the user group generates its own tape recordings.

3. Education about Speech Research. A rather high percentage of the user groups are fairly new to the field of speech. Consequently, as a by-product of their visits to the Laboratories, they have an opportunity to become better acquainted with the entire program of research underway there. Many have taken advantage of that opportunity.

Six senior scientists visited the Haskins Laboratories on September 19-21, 1979, and discussed with Haskins staff the activities supported by this contract. Without exception these reviewers recommended approval of the request for three years of additional support. There was general agreement that the service and assistance provided by the contract have been helpful to many investigators, most of whom would not have been able to otherwise conduct their proposed research. There was also general agreement that there will remain for some years a strong need for the type of research provided through this contract.

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Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD-7-2837

Contract Title: Prototype Test of Decoding Skills

Contractor: Research Foundation for Mental Hygiene, Inc., Albany, N.Y.
Dr. Ellis Richardson

Money Allocated: No FY 1980 Funds

Final Report: The purpose of this contract was to develop a battery of tests that could be used to determine a child's competence in various decoding skills essential to proficient reading. With NICHD's support, a Decoding Skills Test (DST) was developed by the Long Island Research Institute. The initial purpose in developing the DST was to provide a "detailed measurement of decoding skills which could be used in research on developmental dyslexia." Since the final product does provide such detailed measurement of decoding skills and thus is diagnostic in nature, an obvious secondary purpose emerged during the course of its development: To provide a diagnostic-prescriptive instrument which could be used in the evaluation of, and program planning for, children in need of remedial help in reading.

The Decoding Skills Test developed by this contractor is "criterion-referenced" in that its contents have been derived from a wide variety of classroom materials currently in grades 1 through 5. The battery consists of three subtests: Basal Word Recognition (Subtest I), Phonic Decoding (Subtest II), and Oral Passage Reading (Subtest III). The battery was not intended to include a measure of comprehension ability since the primary emphasis in research on reading disability is on the word recognition component of reading. As a function of its diagnostic-preventive role, however, a "simple recall" comprehension supplement was added to the DST so that gross deficiencies in a child's ability to comprehend might be detected.

Staff believes that this test will ultimately be of direct usefulness in the diagnosis and remedial treatment of children with reading disabilities. The contractor has been encouraged to publish this test as soon as possible.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD-7-2836

Contract Title: Prototype Test of Decoding Skills

Contractor: Bolt Beranek and Newman, Inc., Cambridge, Massachusetts
Drs. Ann M. Rollins and Raymond S. Nicherson

Money Allocated: No FY 1980 Funds

Final Report: The purpose of this contract was to develop a test battery that could be used to identify decoding problems that might underlie general reading disabilities. The test battery was to be designed for use with individuals; its administration was to take not more than 30 minutes and to require no special instrumentation. The battery was intended to be used by researchers for the purpose of selecting and screening subjects for studies of reading.

The final battery of tests was developed over a period of 27 months. During that time, the tests were administered to more than 300 students selected from three public school systems in the greater Boston area. Several candidate tasks were tried over the course of the project, not all of which were included in the final battery.

The underlying conceptual framework required that the test be able to identify children whose mastery of particular reading-related skills was not commiserate with their overall level of reading proficiency. That is, the test would be concerned not with the rate, but the balance of component skill acquisition. The final version of the test is comprised of nine tasks: 1) Word recognition, 2) Irregular words, 3) Pseudoword captions, 4) Frequency, 5) Find-the-e's, 6) Approximations (This task was used as a measure of automaticity), 7) Imbedded words, 8) Spaceless sentences, 9) Context. The only major change in the test battery since the last Annual Report was the dropping of the "Jabberwocky" task. The remaining tasks were, however, re-ordered so as to intersperse those that required much continuous reading with those that did not. In addition, minor changes were introduced for a few of the tasks.

The results of the research that produced the test battery demonstrated that the battery can be administered to children with severe reading disability of the type typically classified as dyslexia. It would appear that with careful administration, the tasks are nonthreatening and interpretable results can be obtained. The average time required to administer the test is approximately 30 minutes.

Staff believes that the test, or some derivative of it, will ultimately be directly useful in the diagnosis and remediation of reading disabilities.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1-HD-8-2824

Contract Title: Neuropsychological Studies of Reading Disabilities

Contractor: David Galin, M.D. and Charles D. Yingling, Ph.D.
University of California San Francisco, San Francisco, CA

Money Allocated: $350,000

This contract research program is one of several activities in a program of research on reading disabilities and developmental dyslexia. The immediate objective of this contract research program is to evaluate information processing characteristics in a group of reading disabled children and matched controls utilizing quantitative methods of brain electrophysiology;neuropsychological tests, and psychometric tests. Specific attention is being given to interhemispheric brain functions in the processing of simple and complex stimuli in the visual, auditory and tactile sensory modalities. A special effort is directed toward the evaluation of vestibular-cerebellar mechanism as they may relate to disorders of information processing as reflected in reading disabilities and learning disorders. The ultimate objective of this research program is to develop a parsimonious neuropsychological test protocol for the early detection and identification of potential reading disabilities in the pre-school and/or pre-verbal child. This contract research program was initiated on September 29, 1978, and has received support through March 31, 1982.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD 92831-01

Contract Title: Childhood Antecedents of Habitual Cigarette Smoking

Contractor: University of Minnesota, Minneapolis, Minnesota
Maurice Mittlemark, Ph.D.

Money Allocated: No FY 1980 Funds

The purpose of this contract is to help identify the conditions that lead to habitual cigarette smoking and those that lead to continued non-smoking among adolescents. The study design focuses on adolescent age groups where the transition from occasional (experimental) smoking to regular (habitual) smoking is most likely to occur. The results of this study will be useful for developing programs to reduce the incidence of smoking among adolescents.

In the design of the present study a cohort of 2500 adolescents (500 from each grade of grades 7 - 11) are being followed for two years. They will complete a self report questionnaire (Minnesota Smoking Survey) 4 - 5 times during the course of the study, and three biochemical tests (alveolar air samples for carbon monoxide, saliva thiocynate, and cotinine determination) will be used to validate the self reports. Parents and teachers will complete other questionnaires.

First Year Progress

Because OMB clearance was delayed, it was not possible to initiate the full cohort study immediately. Instead, the contractor modified his procedure to test 400 of the cohort students in the 6th grade (in May 1980, following OMB clearance approval); in September they will then become part of the larger cohort of 2500. Thus, this small group ultimately completes the questionnaire 5 times instead of 4. The transition from elementary school to junior high school is thought to be another critical point in terms of developing the smoking habit.

During the first year of the study, the contractor initially conferred with NICHD staff and submitted copies of all questionnaires for OMB clearance. The contractor also made contacts with the appropriate school system, trained his staff in data collection and management, and began development of the cotinine analysis procedure. In the last quarter questionnaires, saliva thiocynate, and carbon monoxide data were collected for approximately 400 6th grade students; preliminary analysis was also completed. Detailed schedules are now being developed for data collection for 2500 students in grades 7 - 11, to begin in September 1980.

NICHD GRANTS AND CONTRACTS, ACTIVE DURING JULY 1980
Human Learning and Behavior Branch

Funds (thousands)

	Total		Total Research						Fellowships (incl. T32)	
			Total		Grants (incl. RCPs)		Contracts			
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	124	$11,704	114	$11,034	112	$10,310	2	$725	10	$670
Developmental Behavioral Biology	24	1,650	22	1,592	22	1,592	-	-	2	59
Human Learning, Cognition, Perception, Memory	43	3,436	38	2,869	38	2,869	-	-	5	567
Social and Affective Development	12	864	11	850	11	850	-	-	1	14
Human Communicative Processes	26	3,560	24	3,530	23	3,179	1	351	2	31
Health-Related Behaviors	19	2,194	19	2,194	18	1,820	1	374	-	-

Note: Excludes one scientific evaluation grant for $25,000.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children

Mental retardation ranks among the nation's most pressing social and health problems. Persisting from infancy through maturity, it ranks foremost among conditions of chronic disability. Well in excess of 200 causes of mental retardation have been identified and additional causes are being discovered regularly through research investigations. The costs of mental retardation to the nation in FY 1979 are estimated to approximate $10 billion for care, treatment, and lost productivity. The greatest hope for mastery of the problem lies in its prevention and, when this is not possible, in the promotion of the individual's maximum skills through a wide-range of ameliorative, habilitative and restorative procedures.

The NICHD, within the NIH, has primary responsibility for research and research training concerned with mental retardation. This interest is expressed categorically through the Institute's Mental Retardation and Developmental Disabilities Branch (MRDD) of its Center for Research for Mothers and Children (CRMC). The MRDD Branch supports research into the biological, behavioral and social processes which contribute to, or influence the development of, retarding disorders. Of primary concern are studies concerned with finding causes and means for preventing mental retardation. Support for research pertaining to prevention of mental retardation also derives from activities of other branches of the CRMC. The Institute employs research grant mechanisms, supports special research facilities as the Mental Retardation Research Centers (MRRCs), disseminates scientific and public information, and provides contract support for research to accomplish its goals. The Institute's research programs and resources provide research knowledge and understanding applicable not only to mental retardation but to other closely related developmental disabilities as well.

Research into the causes, means of prevention, and methods for the amelioration of mental retardation serve many of the research requirements of developmental disabilities including autism, epilepsy, cerebral palsy and special learning disabilities. Children with these conditions often present symptoms of mental retardation and, with the exception of special learning disabilities, mental retardation is frequently associated with the other disabilities.

These developmental disabilities are a frequent consequence of reproductive casualty resulting from genetic defects, disorders of pregnancy, complications of birth, and maternal ill health. The Institute, because of its assigned responsibility for research in maternal and child health, has a major research concern for these disorders. All of the Institute's programs, both Extramural and Intramural, make significant contributions relating to these issues.

Mental Retardation Research Centers

The Mental Retardation Research Centers (MRRC's) - 12 in number - were constructed under P.L. 88-164 with the mandate to conduct "research and research training in Mental Retardation and related aspects of human development." Under the provisions of the construction grant award, the centers contracted to use facilities for a minimum of 20 years for these purposes. In average, the centers have now completed about 11 years of this obligation.

The legislative language creating the centers emphasized the need for collaborative, multidisciplinary research to address the complex issues in mental retardation. Early applications for construction grants attempted to satisfy these criteria and most of the centers involve investigators from the major departments and graduate schools of the university. All but one involve the medical school in a prominent role. However, it became apparent that this model was inapplicable to some settings because of marked differences between departments in their level of interest in the problem and scientific talent. Consequently, several more narrowly focused centers were established with potential for indepth studies in some facet of biomedical or behavioral research.

As might be expected, centers are necessarily involved in common areas of investigation. While some overlap is unavoidable, and probably desirable to some degree, various approaches are employed to minimize duplication. The communication network that has been established between centers has proven to be an effective mechanism for minimizing excessive overlap of effort and promoting a cohesive national research effort.

RESEARCH HIGHLIGHTS

From its inception, the MRDD Branch has placed emphasis on developing a strong program of genetics research. Added impetus to this development occurred in FY 1978 when both the House and Senate Appropriations Committees included language in their reports urging the Institute to increase its research in genetics, specifically stipulating research in Down syndrome. Data supportive of continued emphasis on genetics/Down syndrome research are compelling and advances realized and expected from Branch contract and grant supported research are encouraging.

An Interregional Cytogenetic Registry was established with contract funds at the University of Oregon Health Sciences Center in Portland. Established with the objective of designing and maintaining a registry of clinical and cytogenetic information, the registry will be instrumental in providing better health care delivery. These goals can be achieved directly through registry use in the delivery of genetic services as diagnosis, counseling, and follow-up, and indirectly as an aid to genetic research.

To these ends, registry data can be utilized to provide information on morbidity, etiology, recurrence risk estimates, frequency of break points, genetic linkage and gene mapping, karyotype/phenotype correlation, mutation rate estimates, genetic counseling, health care needs and delivery.

With the collaboration of five additional and widely dispersed cytogenetics laboratories, the registry design has been successfully accomplished. Standards of uniform recording and reporting of data have been established which have been shown to be valid and reliable across collaborating laboratories. A manual has been developed which is finding broad acceptance by laboratories across the country and abroad.

A major deterrent to a fuller application of cytogenetic knowledge has been the time and effort required to perform chromosome analysis in the laboratory. This challenge has been met through the development of automated procedures for chromosome preparation and karyotyping. This work has been accomplished through use of three separate but related contracts.

A semi-automated system for chromosome preparation has been successfully developed by the contractor at the City of Hope Medical Center, Duarte, California. This system offers major advantages over other systems including (1) ability to process large numbers of specimens; (2) uniform treatment of all specimens; (3) ability to process controls and replicates in same tray; (4) strict control over specimen identification; and (5) reduction in cost and time of processing.

A stand-alone automatic karyotyping device has been developed under contract by the Jet Propulsion Laboratory in Pasadena. Operation of the automated system provides for four major activities, including (1) slide search (that is, selection of metaphase spreads); (2) scanning; (3) spread analysis; and (4) generation of a karyotype. This device, when linked operationally with the semiautomated system for chromosome preparation, provides a complete automated system capable of meeting the burgeoning needs for chromosome analysis in clinical genetics and cytogenetic research.

The complete system is now undergoing field testing and updating under a contract let to Northwestern University. This testing and upgrading of the complete system will enchance its capability by incorporating recent technical and scientific developments in cytogenetics and image processing, thus making the system more versatile and responsive to the cytogenetic community it is intended to serve.

With an incidence of about 1 per 700 live births, Down syndrome is the most common and readily identifiable genetic condition characterized by mental retardation. Despite its recognition as a distinct clinical entity, and the first condition to be associated with chromosomal defect, several fundamental issues concerning this issue continue to elude scientific explanation.

Research restraints on use of human subjects have prompted the Branch to turn to the development of animal models holding promise for providing understanding of the mechanisms involved in Down syndrome. Here, research advances have demonstrated capability for systematic study of trisomy using animal models.

Fragile X Syndrome

Previous studies on the prevalence of mental retardation were based on institutional records, and the noted excess of male retarded individuals was considered to reflect differential sex selection in institutional admission policies. A study which checked for a selective factor for male institutionalization failed to show a bias against male admissions. In addition, recent community surveys showed an even greater surplus of male retardates in the community than in institutions. Among both institutionalized and non-institutionalized retarded children there is an excess of approximately 50% males. Whereas in the past social and behavioral variables were the reasons given for a higher prevalence of mental retardation in males, more and more studies are now being pursued to ascertain the biological reasons to explain this phenomenon.

It has been estimated that X-linked forms of mental retardation were responsible for about 20% of all cases of mental retardation in the I.Q. range 30-55. Five percent of the moderately retarded may be in the fragile X subgroup of familial X-linked mental retardation, making this the most frequent genetic cause of mental retardation in males, and second only to Down syndrome in the association of mental retardation with a chromosme abnormality.

The spectrum of clinical manifestation of the syndrome is being determined by a number of investigators. The emerging phenotype seems to include several of the following signs and symptoms: mental retardation, which is variable in severity from moderate to profound, verbal and auditory deficiencies, lack of fine motor control, hyperactivity, macrocephaly, prognathism, large malformed ears which are frequently low-set and macro-orchidism. Whether or not the female carriers of the fragile site are also clinically affected is still undetermined.

Research emphasis is designed to optimize the conditions for detection of the marker X chromosome by a variety of alterations in the culture conditions. Institute-supported investigators failed to confirm published reports that methotrexate in combination with media deficient in thymine and folic acid enhance the probability of demonstrating the marker X chromosome.

Fibroblasts from obligate carriers and affected males have been cultured successfully, demonstrating the expression of the marker X chromosome. These findings provide a basis for reliable diagnosis of carriers and for improved detection of a preventable form of mental retardation through prenatal diagnosis.

Diet Discontinuation in Phenylketonuria

A Collaborative Study of Children treated for Phenylketonuria (PKU) has been conducted in 15 medical centers across the United States since 1967. The study includes 140 PKU children who were treated from near birth to 6 years of age. At age six, with the consent of the parents, each child was randomly assigned to one of two groups, either to continue on a phenylalanine-restricted diet, or to discontinue and be free to eat a normal diet. The protection against bias afforded by randomization may have been lost since 22% of the parents chose not to accept their children's assignment.

The average I.Q. of the group of patients in whom diet was discontinued at different intervals after age six has been at the same level from 6-10 years (age 6=98.5; age 7-97.8; ages 8 and 10=97). However, these children have had significantly more problems in behavior and social adjustment, and are not achieving academically as well as the much smaller group of children in whom the diet was continued. No major adverse sequelae of dietary discontinuation such as the precipitation of a convulsive disorder, serious behavioral reaction, or profound deterioration in intellectual function has been observed.

As a group, the patients who remain on the diet have an I.Q. which has reached 10 points higher than the group that has discontinued the diet. This finding is a continuation and confirmation of previous data which have demonstrated a positive correlation between higher I.Q. and low index levels of dietary control. No evidence has been produced by this study to document the concern that continuation of diet into the school years produces significant psychological difficulties.

It should be emphasized that the study is still in progress and the sample size upon which the foregoing observations were made are still inadequate to formulate definitive conclusions. Of the 127 total active cases, 120 have reached the age of 7, 90 are now 8 years, and only 42 have reached the age of 10.

Dietary Treatment of Urea Cycle Enzymopathies

Previous therapeutic approaches to patients with urea cycle enzymopathies have been designed to reduce the requirements for urea synthesis by quantitative and qualitative manipulation of dietary protein, amino acids, or their nitrogen free analogs. Success of these approaches to treatment of conditions associated with nitrogen accumulation have been limited, with death usually occurring in infancy among patients with urea cycle enzymopathies.

Scientists from the Johns Hopkins University have developed a new method of treatment by providing patients with nitrogen accumulation, a new pathway for detoxification and renal excretion of otherwise toxic nitrogenous substances. Benzoic acid, or its sodium salt, when given by mouth or intravenously, is rapidly converted to hippuric acid after conjugation with glycine. Hippuric acid is rapidly excreted in the urine. The net effect is the excretion of nitrogen by a new pathway with a consequent decrease in the amounts of toxic nitrogenous substances that accumulate in the body. The same mechanism

follows the administration, either orally or intravenously, of phenylacetic acid or its sodium salt. These substances are conjugated with glutamine and are converted to phenylacetylglutamine and rapidly excreted in the urine.

This new approach can be used in the treatment not only of the urea cycle enzymopathies but other conditions that are characterized by nitrogen accumulation, such as uremia, portal systemic encephalopathy, and Reye syndrome.

Fetal Alcohol Syndrome

The abnormalities associated with the fetal alcohol syndrome involve a variety of organ systems, and are manifest as growth deficiency, a characteristic cluster of facial stigmata, and central nervous system dysfunction. The latter, characterized mainly by mental retardation, is one of the most common and serious problems associated with ethanol teratogenicity.

A follow-up study of 135 children exposed prenatally to varying amounts of ethanol has been completed by University of Washington investigators. The data obtained include: (1) direct behavioral observations of four year old children in their homes; (2) observation of these children at five years of age in structured school-like settings; (3) minor physical anomaly assessment of these chilkdren and their mothers; (4) measurement of the quality of home stimulation; (5) evaluation of maternal personality, current drinking and smoking habits, and family functioning; (6) assessment of children's performance on preacademic tests, and (7) assessment of postnatal growth and health of the children. Preliminary results indicate that maternal social drinking during pregnancy has small but significant long term effects on children's behavior, particularly on attention span and fidgetiness. These observations extend earlier findings which suggest a dose-response curve for intrauterine alcohol exposure. The effects on offspring range from birth weight decrement and functional deficits at the lower doses to a full blown picture of fetal alcohol syndrome. A safe level of alcohol use during pregnancy has not been established, although reduced birth weights have been found at levels corresponding to about two drinks per day on the average.

The mechanisms by which the teratogenic effects of alcohol are produced remain unclear. The question of whether it is the direct effect of alcohol on morphogenesis or the effect of a breakdown product of alcohol, such as acetaldehyde, is now being evaluated by the Seattle investigators.

Environmental Effects

Mercury:

Methylmercury intoxication in Macaca mulatta monkey has produced cerebral palsy and mental and physical retardation in infants of females who were given subclinical doses of methylmercury during pregnancy. These results are the first successful attempts to produce this model, and show promise

for an important experimental model to investigate cerebral palsy and mental and physical retardation due to congenital exposure to neurotoxic agents.

Lead: Classroom Performance in Low Level Lead Exposure:

Investigators at the Children's Hospital Medical Center, Boston, classified children's lead exposure by measuring lead in shed teeth. They demonstrated in 1979 that children with elevated dentine lead levels, who were considered asymptomatic, performed significantly less well on a number of psychological measurements (WISC-R, auditory processing and attention). Teachers who were blind to the child's lead level, reported that high lead children were more distractible and less able to follow directions.

This year, they followed up a sample of the same subjects, now in grades 4 to 6. Children were observed in class at four different times, and their classroom performance sampled at 7-second intervals and classified as being on or off task. A relationship between dentine lead level and amount of time off-task was found. In addition, teachers, again blind to the children's lead level, reported more disordered classroom behavior in high lead subjects. High lead subjects also rated significantly higher on a conduct disorder scale of a behavior problem checklist, and a significantly higher proportion of high lead subjects required special services (remedial reading, language or math help) from their school.

These preliminary findings support the conclusions derived from the earlier data obtained in the psychological laboratory. They indicate that lead exposure at low dose has demonstrated effects on the child's classroom performance.

Malnutrition

An investigator from New York University is trying to determine how protein deficiency during development modifies the organization of neuronal circuitry of the cerebellum. Rat offspring of maternally malnourished dams (8% protein experimental and 25% protein control diets with normal calorie intake for both groups) were continued on the maternal diet after weaning until 60 days of age. At this age, the animals were sacrificed and the cerebellar circuitry was quantitated for layer volume, cell numbers, synaptic densities, and size of synapses and myelinated axons. Gestational protein malnutrition did not alter the number of divisions for Purkinje cells. Gestational protein levels appear to be compensated <u>in utero</u> by utilized maternal body protein.

Quantitation of number and size of synapses revealed an inverse relationship between the number of contacts on each Purkinje cell and their size; as the number of afferents decreased, the size of synaptic contacts increased. It is concluded that this inverse relationship is due to a constant total contact area being maintained by the Purkinje cell itself. The remaining afferent contacts increased their size due to a shift in junctional macromolecules.

Volume of myelinated layer was severely affected in both male and female rats. Decreases in both myeline thickness and axon diameter accounted for a major part of the reduction in white matter volume. Myelin thickness was reduced two-fold that of axon diameter.

This study shows that circuitry is modified when some neurons (granule cells) are not allowed to develop because of protein malnutrition. The direct effect is on the neurons that divide postnatally. For the human, a question remains as to whether division of neurons in utero are entirely protected since all neurons are formed before birth. This study shows that at least one type of cell, Purkinje cells of the cerebellum, which divide in utero, is not affected under the 8% protein diet in rats.

Neuronal Plasticity

Using a special electron microscopy method, an investigator from the University of Chicago Mental Retardation Research Center has demonstrated that infants 1 to 2 years of age have more complex sets of cortical brain cell interconnections and 50 percent more synapses than adults. It was previously thought that synapses in the brain increased with age. In this study of normal human brains, it was demonstrated that the number of synapses actually decreases between ages 2 and 16, remains constant from ages 16 to 72 years, and shows a slight decline in the brains of those aged 74 to 90.

The full significance of the apparent overproduction of synapses during infancy is not totally understood. This initial overproduction of synapses may be an anatomic basis for neuronal "plasticity." If the brain has an excess of interconnections at the time of injury, brain signals may be rerouted, perhaps explaining why the immature brain can sometimes recover from injury more completely than the brain of an adult.

Aniridia-Wilms' Tumor Syndrome

Two childhood cancers, Wilms' tumor and retinoblastoma, have recently been linked to very small congenital chromosomal deletions. Both can be inherited (autosomal dominant), but often they occur sporadically. Patients with Wilms' tumor often also have what is called the AGR triad, a syndrome consisting of aniridia, genitourinary defects, and mental retardation. Both Willms' tumor and aniridia are rare conditions; the incidence of the former is 1/10,000 to 15,000, and the latter is 1/50,000 to 100,000. The two conditions occur together much more often than by chance. Among the 400 patients with Wilms' tumor, six also had aniridia, a ratio of one aniridia to 73 tumors. Patients with the Wilms' tumor-AGR triad have been shown to have interstitial deletion of a small segment of the short arm of chromosome 11. Application of refined staining techniques make it possible to demonstrate subjects with balanced chromosomal rearrangement, enabling scientists to detect the carrier state, making Wilms' tumor one of the few instances in which predispostion to a particular neoplasia can be predicted by prenatal diagnosis.

Wilms' tumor-AGR triad exemplifies the relationship between oncology pediatric research, mental retardation and congenital malformations where territorial distinctions are artificial and vague.

NICHD GRANTS AND CONTRACTS, ACTIVE DURING JULY 1980
Mental Retardation and Developmental Disabilities Branch

Funds (thousands)

Health Area	Total		Total Research						Fellowships (incl.T32,T35)	
			Total		Grants (incl. RCPs)		Contracts			
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	146	$28,828	126	$27,106	122	$26,285	4	$821	20	$1,722
Etiology	71	9,807	62	9,012	61	8,821	1	191	9	794
Epidemiology	5	677	3	536	3	536	-	-	2	142
Diagnosis and/or Evaluation	43	6,902	34	6,116	31	5,487	3	629	9	786
Prevention	3	901	3	901	3	901	-	-	-	-
Amelioration--Education	7	1,245	7	1,245	7	1,245	-	-	-	-
Amelioration--Medical Treatment	2	651	2	651	2	651	-	-	-	-
Other (includes Implementation Grants)	15	8,645	15	8,645	15	8,645	-	-	-	-

Note: Excludes one scientific evaluation grant for $105,000.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1-HD-7-2847

Contract Title : The Impact of Foster Family Care in the Development and Behavior of Mentally Retarded Children

Contractor : Regents of the University of California at Los Angeles

Money Allocated: FY '80 - $14,185

The purposes of this contract are to determine:

1. Characteristics of children most likely to profit from foster family care.

2. The kind of socialization and developmental experiences afforded to such children.

3. The qualifications and characteristics of foster parents conducive to successful placement.

4. The most appropriate and effective roles of foster family members, natural parents and agency workers.

5. Special considerations and community supports needed to adapt foster family care to needs of the retarded.

6. Utilization of foster family care for long term placement.

This project is about to enter the final quarter of proposed work. Data collection is virtually complete. Data analysis has begun and the remaining time will be devoted to data analysis and reporting. The work is on schedule and is being conducted in an exemplary manner, including the presentation and/or publication of preliminary findings in ten separate reports.

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October 1, 1979 through September 30, 1980
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1-HD-32713

Contract Title : Coordination of Regional Cytogenetic Registries

Contractor : University of Oregon Health Sciences Center

Money Allocated: FY 1980 - $270,000

This contract was initiated to develop a common format for the collection of clinical and cytogenetic information; to develop a manual of procedures to standardize the selection of material for analysis and for maintenance of quality control; and to develop a phenotype coding system. The registry is designed to maintain a record of genetic and clinical information which will help provide better health care services, and to provide a data base for research and epidemiologic studies.

Data base presently includes information on 30,396 patients. On-line computer entry has been replaced by batch entry from key-punched tape. A new program has been developed which prevents duplicate entries on the same subject as a result of multiple evaluations. Quality control of data input has been initiated; the principal investigator visited each of the collaborating laboratories, recoded about 25 cases from each center which will be compared with the data currently in the registry. The participating laboratories are located in Portland, Memphis, Albany, Indianapolis, and New Haven.

The six research projects which were initiated last year to demonstrate the feasibility of retrieving registry data will be completed during the current contract year which ends in January 1981.

A microfiche system was initiated this year and has proven to be an economical and useful tool for data management.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1-HD-8-2855

Contract Title : Field Testing for Chromosome Preparation and Karyotyping Device

Contractor : Northwestern University

Money Allocated: FY 1980 - $275,000 (est.)

The objectives of the contract are to carry out a comprehensive evaluation and testing of an automated karyotyping device which was developed at the Jet Propulsion Laboratory, and a semi-automated system for chromosome preparation which was developed at the City of Hope Medical Center.

The specimen preparation device has undergone extensive evaluation and subsequent modifications. A series of trials showed that hypotonic incubation time and centrifuge speed made no difference, while blood volume and phytohemagglutinin concentration influenced the number of growing cells, metaphase quality and mitotic index. The Gam-Rad staining system is performing as well as the manual method. The software has been redesigned for improved operation. All routines are being written in FORTRAN for greater flexibility and ease of modification. A Teleray CRT terminal has replaced the DEC writer as the primary operator's console. The error rates for bar code reading and slide jamming have been reduced significantly. The performance of the automated system was compared with the manual method in terms of time, cost, and effort. The automated system is superior in preparation time especially when 48 cultures per day are processed, it costs less to operate by about 1/3, and less effort is expended when more than 12 cultures are run.

The investigators continue to experience difficulties with the metaphase finding and automated karyotyping device. Part of the problem stems from the fact that the rapid rate of evolution of computing technology had rendered portions of this machinery semi-obsolete. It is anticipated that considerable difficulty will be encountered in replacing the original hardware, which were "frozen" using 1973 technology, with more advanced and contemporary machinery. Budgetary constraints might make it difficult to up-date the automated device to the satisfaction of the cytogenetic community it is intended to serve. The contractor will evaluate the relative economics of replacement with new equipment, with the consequent low maintenance costs, versus simple repair and associated high maintenance costs and suboptimal performance.

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1-HD-8-2858

Contract Title : Development of An Animal Model for Genetic Disorders with Emphasis on Trisomy 21 (Down Syndrome)

Contractor : University of California, San Francisco

Money Allocated: FY 1980 - $150,000 (est.)

Aim #1: Expansion of existing mouse colonies--The mouse colonies in Lubeck and San Francisco have undergone major expansion. The composition of the San Francisco colony is determined by the needs of the work in progress.

Aim #2: Isolation of new metacentric chromosomes--Three trips to northern Italy were made to trap mice possessing Rb translocations. Of 70 animals trapped in 17 locations, 52 carried a total of 105 Rb translocations.

Aim #3: Analysis of the frequencies of production of various trisomies and characterization of segregation patterns--Seven metacentrics were investigated with regard to metaphase II counts in spermatocytes and oocytes. Malsegregation rates are always higher in female progenitors than in males. Similar observations in man have to be interpreted as corresponding to a general biological, though unexplained, phenonmenon.

Aim #4: Development of techniques for freezing embryos--Success has been achieved in freezing embryos from laboratory strains of mice and in one case with an embryo from a translocation carrying congenic mouse. However, success has not yet been achieved in freezing embryos from feral mice because of problems in inducing superovulation in wild mouse strains.

Aim #5A: Mapping of human chromosome 21 homologous loci on the mouse genome and related gene dosage studies--Utilizing somatic cell genetic techniques, the investigators have clearly demonstrated that SOD-1 and IfRec are located on mouse chromosome 16. The mapping of GARS has been hampered by the lack of an appropriate electrophoretic system for identifying the enzyme.

Aim #5B: Investigation of effects of mouse trisomy 16 on cellular function--Two types of trisomy/normal chimeras, radiation and aggregate, have been produced.

Aim #6: Analysis of the effects of aneuploidy on development--The timing of the lethality of the trisomic fetuses or newborns has been well established for each of the trisomies.

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October 1, 1979 through September 30, 1980
Office of the Director
Epidemiology and Biometry Research Program

The Epidemiology and Biometry Research Program (EBRP) conducts epidemiological and biostatistical investigations of disorders of mothers and children, of perinatal mortality and morbidity, of infant nutrition and abnormalities of human growth and development. In addition the Program provides expert biostatistical advice to intramural scientists and to the extramural programs of the Institute. Epidemiological consultation is provided upon request to programs of the Center for Research for Mothers and Children, the Center for Population Research, and the Office of the Director, NICHD.

Several staffing changes have occurred during the past year. Mr. Nathaniel White was transferred from the Biometry Branch to the Office of the Director to serve as special assistant to the Director in the development of an effective liaison to a perinatal research epidemiology unit in the District of Columbia. Dr. James Mills, a Staff Fellow in the intramural program of NICHD, is assigned to EBRP and is pursuing several epidemiological investigations with EBRP Staff. Dr. Susan Harlap, Associate Research Professor, Hebrew University, Hadassah Medical School, Jerusalem, served as a visiting scientist from July through October, 1980, and pursued analysis of data from the Jerusalem Perinatal Study.

Active recruitment is still in progress to seek a Branch Chief for the Epidemiology Branch.

Several new research initiatives have been considered. Among them investigations leading to a better understanding of the nature of the perinatal risks associated with pregnancies in very young teenagers in an effort to separate possible biological effects from social, demographic, or perinatal care variables. Data are being developed to address these questions with the help of the New York City Health Department, data from the collaborative perinatal project and perusal of linked births-deaths records from several states in the U.S. for the periods 1967 through 1973.

In collaboration with Dr. Godfrey Oakley from the Center for Disease Control and Dr. Rosalyn Epps, the Commissioner of Health from the District of Columbia, we have been discussing the merits of a perinatal research epidemiology unit in the District of Columbia to pursue research regarding the high infant mortality rate in the District of Columbia and of factors associated with pre-term delivery and other high-risk pregnancies. While the scope of the research activity is still under discussion, CDC has already assigned an EIS officer full-time to the unit. EBRP is planning to involve itself directly in the research activities of such a unit and provide data processing support. It is hoped that this effort will lead to extensive field research about critical issues associated with perinatal risks on the part of EBRP in collaboration with the other interested parties which has application beyond the District of Columbia.

The feasibility of a clinical trial of intrapartum electronic fetal monitoring was explored with the help of consultants and also by contacting about 50 leading perinatologists in the United States and Canada. A majority of the individuals consulted questioned the ethics of a randomized clinical trial involving high-risk pregnancies and were fearful of the medical-legal implications in their own community if high-risk women, through randomization, were denied electronic fetal monitoring. A clinical trial involving low-risk or normal pregnant women requires sample sizes in excess of 50,000 and was, therefore, considered not feasible because of the considerable cost involved.

The Program provided support to the Institute's effort in the development of a five-year-plan for research by taking responsibility for the compilation of resource data regarding the public health significance of research and research needs in the major program areas of the NICHD.

Dr. Keller and Dr. Berendes provided input into the NHIS Child Health supplement, 1981, and developed on outline of the analysis especially of the sections dealing with the Prenatal Health Habits and The Birth Episode.

The Director continues to serve on the Advisory Committee of the WHO Special Program for Research in Reproduction. In addition, he was chairman of a scientific group of the World Health Organization which met in Geneva from December 10-14, 1979, to develop a state of the art report on the effects of steroid sex hormones on fetal development and infant health.

The Director also serves on the Fertility and Maternal Health Drugs Advisory Committee of the FDA and the Advisory Committee to explore the need for a broad scale epidemiological perinatal study by the Center for Disease Control.

He participated by invitation in a conference sponsored by the Office of Smoking and Health of the Office of the Assistant Secretary for Health entitled "Research Needs of Low Yield Cigarettes" which was held June 9-11, 1980, in Bethesda, Maryland. He is a member of the NIH Clinical Trials Committee.

He lectured on numerous topics during the past year at Johns Hopkins University in Baltimore, Howard University in Washington, D.C., Columbia University and the University of Minnesota in Minneapolis.

The Section on Systems Design and Data Processing continued its mission of developing and implementing systems for handling the many studies in the Epidemiology and Biometry Research Program area. System staff members continue to provide technical consultation in the discussion of and to collaborate in the design of studies, questionnaire development, data organization, data collection, and to do preliminary programming to answer initial questions arising from study development.

Data collected on children and mothers involved in the studies are varied and massive. The following paragraphs give sketches of the variety of work performed by the Section in support of the other branches in the research area.

This past year has been especially productive in the design of an automated data entry system for the clinical trial of early onset cystinosis using a new drug cysteamine. A system was designed such that a data base could be created simply by entering data directly from a subject's interview form. The system, by prompting, allows an individual to enter data without the data having been previously placed on a coding sheet. The system edits, updates, and analyzes the data with a simple response to a question. The system is the pilot procedure for subsequent new studies in the program area.

The Section on Systems D e sign and Data Processing has continued to provide support in the form of data base maintenance, tabulations and graphing for the following studies:

1. A clinical trial that makes use of phototherapy in the treatment of neonatal hyperbilirubinemia. A data base of over 18,000 records to date have been created;

2. A study of breast feeding in relation to the child's health was performed utilizing data obtained from a Pima Indian Population;

3. A study of high infant mortality in a nearby metropolitan area designed to determine the relationship of environmental factors to the cause of death for infants who died in the first year of life;

4. Studies using information from linked birth and death certificates for years 1967 through 1973 for several states in the U.S.;

5. A study to determine obesity in early childhood;

6. A study of the tendency to repeat birth weight and gestational age in successive births;

7. An investigation of crown-heel length in physical growth; and

8. Neonatal mortality in the District of Columbia.

Currently, the staff is providing technical consultation in the discussion of questionnaire development, data collection and data organization for a new study on mothers at risk from diabetes.

Mr. Nathaniel B. White's activities are summarized as follows:

Consultations:

Dr. Donald Mattison (PRB, NICHD) - The manuscript for the first paper entitled "The Effect of Benzo (a) Pyrene on Fertility, Primordial Oocyte Number and Ovarian Response to Pregnant Mare's Serum Gonadotropin" has been completed. Submission to a journal awaits passage through the NICHD review process. I am currently involved with Dr. Mattison in the "PAH Breeding Study" which will assess the dose dependent interruption of reproductive capability by polycylic aromatic hydrocarbons. Data analysis will be completed by September 30.

Dr. Gere DiZerega (PRB, NICHD) - Analysis for clinical trial has been suggested and will be submitted as part of the proposal to the FDA. Data analysis and computer processing will be conducted at three time intervals; after 10, 25, and 50 (final) cases and controls have been obtained. This is a randomized double blind study which will assess the effectiveness of 32% Dextran 70 in the prevention of the reformation of adhesions following tubal surgery in infertile women. Note: Has been approved by the FDA and I have recently submitted a randomization scheme to be utilized by the 6 collaborating centers.

Dr. Juarlyn Gaiter, Dr. Gordon Avery (Children's Hospital) - The manuscript entitled "Stimulation Characteristics of Nursery Environments for Critically Ill Pre-term Infants and Infant Behavior" was presented by Dr. Avery in St. Julien, France and will be the lead article in the book of the proceedings. Results were presented by J. Gaiter at a meeting in Aspen, Colorado, August 4-6. A second paper is being prepared from the same data to be submitted to one of the psychological journals. The major study which will compare the environments of the RDS newborns with a group of sick non-RDS newborns and also well babies in the data collection mode and will require some preliminary analyses in the near future. Preliminary analysis has begun and abstract will be prepared by September 15, for presentation next April.

Betty Marcus (CFRB, NICHD) - The discriminant analysis of the 2, 6, and 12 month mother-infant interaction data has succeeded in separating the 39 babies into the 5 groups (with only 2 misclassifications) that we produced earlier from 13 month data which was observed during separation and reunion episodes. (A manuscript is in preparation which describes this analysis.) Data has been reworked after changing from 5 to 4 groups; paper will be finalized in early September.

Non-parametric Tests for Spread - Dissertation proposal will be finalized within the next three weeks.

Projects:

County Study of Infant Mortality - Initial maps of county rates of one day, early neonatal, late neonatal, post neonatal, and infant mortality by race and sex have been produced (Aug. 5). Further analysis will follow.

D. C. Epidemiology Unit on Infant Mortality - Did background reading, attended hearings, seminars and held numerous conversations regarding infant mortality in D.C. Obtained file of 1977-78 deaths which was used as basis for National Capitol Medical Foundation Study and we will do some analyses. Series of meetings regarding unit have been attended. (Note: I have agreed to work at least one day a week downtown with Dr. Pleet. Also, Paula Adam will probably work at least 2 days a week.)

Other activities include:

Dr. Fredrickson's Task Force to study the Division of Equal Opportunity and Chairperson, ASA Committee on Minorities.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Office of the Director
Epidemiology and Biometry Research Program

Publications and Talks

Publications:

Harlap, Susan, Shiono, Patricia H., Ramcharan, Savitri, Berendes, Heinz, and Pellegrin, Frederick: A prospective study of spontaneous fetal losses after induced abortions. New Engl. J. Med. 301:677-681. September, 1979.

Shiono, P.H., Harlap, S., Ramcharan, S., Berendes, H., Gupta, S., and Pellegrin, F.: Use of contraceptives prior to and after conception and exposure to other fetal hazards. Contraception 20, No. 2:105-120, 1979.

Bahn, A., Mills, J., Snyder, P., Gann, P., Houten, L., Bialik, O., Hollman, L., and Utiger, R.: Hypothroidism in workers exposed to polybrominated biphenyls. New Eng. J. Med. 302:31-33, 1979.

Mattison, D., White, N., and Nightingale, T.: The effect of benzo (a) pyrene on fertility, primordial oocyte number and ovarian response to pregnant mare's serum gonadotropin. Accepted for publication in Pediatric Pharmacology.

Gaiter, J., Avery, G., Temple, C., Johnson, A., and White, N.: Stimulation characteristics of nursery environments for critically ill pre-term infants and infant behavior. In Stern, L., Oh, William, Friis-Hansen, B. Intensive Care in the Newborn III. Masson Publishing Co., USA, 1980.

Talks:

Berendes, Heinz W.: Risks and benefits of modern methods of contraception. Johns Hopkins University, Department of Pupulation Dynamics, Baltimore, MD, October, 1979.

Berendes, Heinz W.: The effects of sex hormones on fetal health and application of epidemiological methods for the study of these problems. Howard University, Department of Pediatrics, Washington, D.C., January 26, 1980.

Berendes, Heinz W.: The adverse health effects of diethylstilbesterol on the male and female offspring. Columbia University, Departmrnt of Epidemiology, New York, NY, February 8, 1980.

Berendes, Heinz W.: The effects of prenatal exposure to DES on the female and male reproductive system and on the application of epidemiological methods to the study of pediatric disorders. The University of Minnesota, Department of Pediatrics, Minneapolis, MN, June 18, 1980.

White, N.: Impact of census undercount. Second Annual Conference on Ethniciry, San Diego, Calif., November, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 00314 Z01-HD-~~0014~~ EBRP

PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

HLA Types and Malformations in Infants of Diabetic Mothers Pilot Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Marvin Cornblath	Spec. Assistant to Scientific Director	IRP	NICHD
Heinz W. Berendes	Director	EBRP	NICHD
Joanna Koppe	Neonatologist, University of Amsterdam		

COOPERATING UNITS (if any)

University of Amsterdam, The Netherlands

LAB/BRANCH

EBRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Diabetes Mellitus is known to be associated with certain HLA types. It is also known to cause malformations in infants of diabetic mothers. This study will examine the relationship between HLA "markers" for diabetes and malformations. The pilot study will examine HLA types of malformed patients, mothers and siblings in The Netherlands to determine whether a formal case-control study is warranted.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 00315 Z01-HD-~~0015~~ EBRP

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Malformations in Infants of Diabetic Mothers in Jerusalem

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Susan Harlap	Visiting Scientist	EBRP	NICHD
Ernest Harley	Supervisory Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)

Hebrew University, Jerusalem, Israel

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.35	.15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of the study is to demonstrate that infants of diabetic mothers have an increased risk of congenital anomalies. This study is unique in that it uses control subjects from the same time and place, and it covers almost the entire population of a defined geographic area. Data from the Jerusalem Perinatal Project will be used in a case-control format.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 0316 Z01-HD-0016 EBRP

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Review of Teratogenic Effects of Maternal Diabetes Mellitus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills Staff Fellow EBRP NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project was to summarize the current state of knowledge of teratogenic effects of maternal diabetes mellitus and to review suggested teratogenic mechanisms. This will result in an article for publication in the medical literature.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 00317 Z01-HD-~~0017~~ EBRP

PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Adverse Effects of Coitus During Pregnancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Susan Harlap	Visiting Scientist	EBRP	NICHD
Ernest Harley	Supervisory Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

EBRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.35	.15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to identify adverse effects, if any, of intercourse late in pregnancy. A case-control approach is being used. Data is retrospectively ascertained from women in the Jerusalem Perinatal Study. Risks of pre-term delivery, low birth weight, stillbirth and neonatal death will be examined.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NO1-HD-0-2839-40-41-42-43

PERIOD COVERED
October 1, 1979, through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Malformations and Fetal Losses in Pregnancy in Women with Juvenile Onset Diabetes Mellitus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Heinz W. Berendes	Director	EBRP	NICHD
Howard Hoffman	Mathematical Statistician	BB	NICHD
Dolores Bryla	Statistician	BB	NICHD
Ernest Harley	Supervisory Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)

Boston Hospital for Women
Cornell University
Northwestern University
University of Pittsburgh
University of Washington

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Epidemiology research program solicited proposals, conducted a technical review, negotiated with acceptable centers, and awarded contracts in the 1978-1979 fiscal year. The collaborating institutions are currently recruiting personnel and organizing to begin recruiting subjects by January 1, 1981. Protocol development will begin with a meeting of principal investigators August 7th and 8th at NICHD. An Advisory Group is being recruited. It will be chaired by Leon Gordis, M.D.

PHS-6040

NICHD Annual Report
October 1, 1979 through September 30, 1980
Office of the Director
Epidemiology and Biometry Research Program

Contract and Collaborative Research

Project Title : Malformations and Fetal Losses in Pregnancy in Women With Juvenile Onset Diabetes Mellitus

Contractors : The Boston Hospital for Women, Cornell University Medical Center, The University of Pittsburgh, Northwestern University Medical Center, and The University of Washington

Money Allocated:

Boston Hospital for Women -	$ 89,087
Northwestern University	98,879
University of Pittsburgh	79,392
Cornell University	107,746
University of Washington	177,855

Objectives: Performing a prospective collaborative study

1. To examine the relationship between maternal diabetic control during organogenesis and malformations in the offspring. To identify, if possible, a specific teratogenic factor or factors in the diabetic metabolic state;

2. To compare early fetal loss rates in women with diabetes and control subjects.

Significance to Biomedical Research and the Program of the Institute: The incidence of malformations in offspring of juvenile diabetic women is believed to be at least twice that of normal women. The early fetal loss rate in diabetic women has not been determined. This study will search for a tertogenic mechanism in diabetes and attempt to determine whether good diabetic control during organogenesis can prevent these excess malformations. In addition, it will be determined whether or not diabetic women have increased losses early in pregnancy.

Proposed Course: The collaborators will complete the study protocol, questionnaire and reporting forms design, and recruiting and organization of personnel by the end of calendar year 1980. Subject recruitment will begin in January, 1981. The project will be reviewed after two years to assess progress and continued support.

Project Officer: James L. Mills, M.D., M.S.

ANNUAL REPORT

October 1, 1979 through Septebmer 30, 1980

Epidemiology Branch
EBRP/NICHD

During the past year there have been no changes in the professional or supportive staff of the Branch. Two summer students have been assigned to work in the Branch and full-time professional staff currently consist of Drs. Keller and Forman, and Mr. Nugent.

Projects in which members of the Branch have or have had primary responsibility during the past year include:

1. The Seasonality of Infant Mortality From Linked Birth-Death Records in North Carolina and Minnesota, 1967-73.

2. Infant Feeding Among The Pima Indians: Time-Trend Factors Associated With Infant Feeding and Its Effect on Child Health.

3. The Epidemiology of Infant Mortality in Baltimore, MD.

4. The Effects of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins.

5. The Geographic Variation of Fetal and Infant Mortality and Low Birth Weight in the United States, 1969-1974.

6. The Relationship of Body Density to Other Methods of Assessing Obesity In Eight Year Old Children and the Association of Obesity With Present and Past Nutritional and Activity Factors and Parental Attitudes.

7. Breast and Bottle Feeding in the United States: The 1979-80 National Natality Survey.

8. National Natality Follow-Back Survey and National Fetal Mortality Survey.

A brief description of these projects is included in the next section.

Additional areas of concern to the Branch staff include the effects of ascending infections during pregnancy on premature delivery, and the relationship between chemical exposures and reproductive outcome. Additional contract requests may be developed in these areas.

Other professional activities of the staff included presentations at the United Nations, Columbia University, the University of Maryland, The Johns Hopkins University, and at national meetings. Staff members have also been involved in several committees of national significance such as the PHS Nutrition Study Group on Refugees (Indochinese refugees) on which Dr.Forman has participated. Dr. Forman has also been involved in the Maternal and

Infant Nutrition Program sponsored by the Department of State's Agency for International Development; in May, 1980, she was a delegate to State Department's United Nations' Mid-Decade Conference on Women held in Washington, DC; and here at NIH, she is the Chair of the Women's Advisory Committee.

Dr. Keller serves as a member of the DHHS, CCERP Sub-committee on Potential Health Effects of Toxic Chemical Dumps where he is involved with the reproductive outcome of victims of the Love Canal. He also acts as consultant to the Scientific Panel, Interagency Work Group on Phenoxy Herbicide and Contaminants (Agent Orange); he is a member of the FDA's Project Advisory Group for Veterinary Adverse Drug Experience Reporting Program; and he is a member of the Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds at the National Academy of Sciences/National Research Council.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	XXXXXXXXXXX PROJECT NUMBER CONTRACT NUMBER: NO1-HD-7-2845-02

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Epidemiology of Infant Mortality in Baltimore, MD

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Project Officer:		Carl A. Keller	Acting Chief	EB/EBRP/NICHD
P.I.	:	Genevieve Matanoski	Professor	Johns Hopkins Univ.
Others	:	Michele R. Forman	Epidemiologist	EB/EBRP/NICHD
		Robert P. Nugent	Res.Assistant	EB/EBRP/NICHD
		Karen Fetterly	Computer Specialist	CS/EBRP/NICHD
		May Chiu	Computer Specialist	CS/EBRP/NICHD

COOPERATING UNITS (if any)
The Johns Hopkins University School of Public Health

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.45	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This contract was designed to study the simultaneous effects of a number of risk factors for infant mortality in a total community. The contractor was let pursuant to an RFP which specified that the study be conducted in a high risk community within 50 miles of NIH so that Branch staff could collaborate with the contractor frequently on a regular basis. The results of this study will be used to assess the relative importance of a number of risk factors for infant mortality which might be incorporated in the design of an intervention program.

PHS-6040

1. Contract Description:

This contract was awarded to Johns Hopkins University School of Public Health on September 30, 1977 for the 2-year period through September 29, 1979 in the total amount of $333,250.00.

An extension of 6 months with an additional $30,114 was approved in September 1979, and an additional 4-month unfunded extension was granted in March, 1980.

The contract is a three-part process and a description and progress are as follows:

i) Case Control Study: Information on all resident infant deaths occurring in Baltimore from November, 1978 to August, 1979 was collected by completion of the contract. A total of 182 deaths occurred of which 145 were able to be located and agreed to be interviewed. Matched and unmatched controls for each case were also selected with 73 matched controls and 242 unmatched controls having completed interviews. Analysis of the data was started in August 1980.

ii) A detailed examination of the causes of death reported on the death certificate and comparison with hospital records and other information on all deaths during a two-year period beginning October 1, 1978. This information is expected to be delivered in August, 1980, and analysis will begin at that time.

iii) An environmental study using demographic and environmental variables to determine the possible relationship of these variables to fetal and infant death rates and low birth weight ratios by census tract for the metropolitan Baltimore area for a 5-year period.

Data collection for this phase has been completed and collaborative analysis is now underway with Monroe Lerner of Johns Hopkins University and Epidemiology Branch staff.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	XXXXXXXXXXX CONTRACT NUMBER: NO1-HD-7-2848-03

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Relationship of Body Density To Other Methods of Assessing Obesity In Eight Year-Old Children and the Association of Obesity With Present and Past Nutritional and Activity Factors and Parental Attitudes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Project Officer:	Carl A. Keller	Acting Chief	EB/EBRP/NICHD
P.I. :	Ruth Huenemann	Professor	U.C., Berkeley
Co-P.I. :	William Bruvold	Assoc.Professor	U.C., Berkeley
Others :	Karen Fetterly	Computer Specialist	CS/EBRP/NICHD

COOPERATING UNITS (if any)
University of California, Berkeley School of Public Health

LAB/BRANCH
Epidemiology

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.20	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The measurement of obesity in children is not a universally accepted process. Circumferences, skinfolds, height, and weight have been used in adults and correlate well with more definitive measurements based on body density or fat content, but the same procedures have not been tested adequately in pre-pubescent children. This project was designed to make simultaneous measurements using all of these procedures, including underwater weighing, and to correlate these with previous measurements on the same children and with food intake histories, activity scores, and parental attitudes toward food consumption.

PHS-6040

1. Contract Description:

This contract was awarded to the University of California School of Public Health, Berkeley, California on September 30, 1977 for a period of 18 months in the amount of $77,331.

The study population consists of all children remaining in a cohort of births to residents of Berkeley, CA in 1969 and on whom anthropometric, food history, activity, and parental attitude data have been collected at one to two-year intervals to the present.

The objectives of this study are to develop a valid method for the measurement of body fat in eight to nine-year old (pre-puberty) children. Practical suggestions for the use of anthropometric measurement of pre-pubescent obesity which can be accomplished with available clinical instruments will be determined. In addition, these measurements will be related to previous eating and activity practices as well as previous anthropometric measurements on the same children to assess the effects of early feeding and activity and the predictive values of early measures on pre-pubescent body fat content.

The results of this study should provide data for more accurate clinical measures of childhood obesity and early predictors for these measures. It is planned to perform data analysis at NICHD.

An extension of 3 months, without additional funds, was granted in order to allow better organization of data to be submitted to NICHD for final analysis. All data tapes have been received with documentation and are currently being analyzed. One of the preliminary findings of immediate interest is that the triceps skin fold thickness is most highly correlated with body fat determinations of all standard anthropometric measures in this young group of children as is the core with post-pubescent children and adults. A final report on this subject will be completed during FY 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00305-03 EB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Geographic Variation of Fetal and Infant Mortality and Low Birth Weight in the United States, 1969-1974

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Carl A. Keller	Acting Chief	EB/EBRP/NICHD
Other :	Karen Fetterly	Computer Specialist	CS/EBRP/NICHD
	Nathaniel White	Math.Statistician	EBRP/NICHD
	Kathleen Thomas	Secretary	EB/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.35	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The assembly of a large number of variables, including pregnancy outcome, has provided data on the marked variability in infant and fetal mortality among different populations in the U.S. The correlation of adverse pregnancy outcome rates with environmental variables will make it possible to assess the relative importance of the various factors associated with these rates in a large population. These data will also provide a basis for the assessment of changes in infant and fetal mortality which may be occurring in some populations.

PHS-6040

1. Project Description:

Approximately 80 variables have been assembled for each of the 3,078 counties in the United States and include data from the 1970 census and other sources pertaining to environmental and geographical characteristics of each county. The file also includes births, fetal and infant deaths, mother's age, parity, and race.

Analysis will include multivariate statistical procedures and description of various factors associated with high and low rate of adverse pregnancy outcomes and should be completed by the end of FY 1981. It is planned to produce a report of these findings in monograph form, including maps.

Analysis using these data was focused on a comparison of adverse pregnancy outcomes in counties with significant chemical and petroleum manufacturing industry with counties of similar size and location, but without such industry. This phase of the analysis was completed in March, 1980 with no significant association found between petroleum and chemical manufacturing industry and adverse pregnancy outcome.

No publication of these findings is anticipated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00306-03 EB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Seasonality of Infant Mortality From Linked Birth-Death Records in North Carolina and Minnesota, 1967-1973

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	:	Robert P. Nugent	Research Assistant	EB/EBRP/NICHD
Others	:	Carl A. Keller	Acting Chief	EB/EBRP/NICHD
		Ernest Harley	Computer Specialist	CS/EBRP/NICHD
		Karen Fetterly	Computer Specialist	CS/EBRP/NICHD
		Maria Keyser	Computer Aide	CS/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.67	.67	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The observed seasonality of neonatal deaths in the U.S. has implications as to the role of environmental agents, e.g., infectious organisms, during pregnancy in influencing pregnancy outcomes. Since seasonality of conceptions may generate seasonality of neonatal death rates, linked birth-death records from North Carolina and Minnesota are being utilized to examine this relationship to that seasonality of death rates can be assessed independently of any seasonal variation in conceptions.

PHS-6040
(Rev. 10-76)

1. Project Description:

The data needed for this project have already been obtained through the LINK program and are part of Branch resources.

The neonatal mortality rate shows an excess of deaths during the first week of life each year in the late spring in the United States. An investigation of the nature of this peak is being made using linked birth-death records so that an account may be made for seasonal variations in conceptions, gestation length, and fetal deaths. Any seasonal variation in infant deaths not due to these factors has implications for variations in infant mortality from environmental causes such as infections.

Analysis is currently nearing completion, and preliminary results were presented in October, 1978 at a national meeting. Of considerable interest is the finding that there is a significant increase in the probability of a pre-term birth and of an early neonatal or late fetal death during July, August, and September of each year in both Minnesota and North Carolina. This seasonality is most dramatic for causes of perinatal death related to infections in mother, fetus, or infant. An evaluation of these findings along with evidence in the literature for the past 20 years is highly suggestive that ascending infections during pregnancy may play a significant role in premature delivery and associated perinatal mortality.

As a result of this project, considerable effort is being devoted to further development of this subject.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00310-03 EB/Inter-agency Clinical Investi. No.78-CH-102 w/NIAMDD

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Infant Feeding Among The Pima Indians: Time-trend Factors Associated With Infant Feeding and Its Effect on Child Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Michele R. Forman	Epidemiologist	EB/EBRP/NICHD
Others :	Ernest Harley	Computer Specialist	CS/EBRP/NICHD
	Norman Kretchmer	Director	NICHD
	Peter Bennett	Chief	EFB/NIAMDD
	Frank Falkner	Director	Fels Res. Institute
	Robert Beren	Med. Records Abstractor	EB/EBRP/NICHD
	Kathleen Thomas	Secretary	EB/EBRP/NICHD

COOPERATING UNITS (if any)

NIAMDD
Fels Research Institute

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The decrease in breast feeding in infants in the U.S. over the past 30 years has been reflected in Native American populations in a shorter period of time. This study is intended to document changes in breast feeding among the Pima Indians living on a reservation in Arizona and to compare the health effects of breast and bottle fed infants over the past 20 years. The availability of centralized health records at the Sacaton Hospital of the Indian Health Service and the relative lack of mobility of the population makes this an excellent source for the study of this issue of current national concern.

PHS-6040

1. Project Description:

The objectives of this study are to determine the trend in breast and bottle feeding over time and the reasons for this trend among the Pima Indians and to determine whether breast feeding compared to bottle feeding is associated with reduced infections, reduced infant mortality, weight gains, and cholesterol levels in children.

The methods being used to meet these objectives include interviewing a sample of 257 women on the Gila River Reservation in Arizona, reviewing medical records kept in the Indian Health Service Hospital, and locating and reviewing vital records on the study population. Development of the questionnaire, interviewer's and coder's manuals has been completed. The survey has also been completed and the analyses are being done at present. Review and abstraction of the medical records was completed during FY 1979.

The following results have been submitted for publication:

Between 1949 and 1977, there was a significant decline in the proportion of women who breast fed. This decline was evident among women age 35-44 in 1978, while women age 30-34 experienced an increase in breast feeding, across socioeconomic strata. Between 1949 and 1963, women of 50-100% Pima Indian descent breast fed significantly less than those of other tribal affiliations, however the influence of tribal descent was reduced thereafter. Parity had a positive association with bottle feeding over time, while family size had a noticeable effect on the association between parity and bottle feeding. The highest proportion of bottle feeding occurred among women with small families before 1963 and among women with large families after 1963 across parity.

The remaining analyses will focus on: the role of pre-and post-natal factors on the trend in breast and bottle feeding as well as comparison of morbidity episodes during the first year of life between breast and bottle fed children.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00311-01 EB

PERIOD COVERED
October 1, 1978 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Breast and Bottle Feeding In The United States: The 1979-80 National Natality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Michele R. Forman	Epidemiologist	EB/EBRP/NICHD
Others :	Ernest Harley	Computer Specialist	CS/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Participation in the National Natality Survey of the NCHS will provide data on current breast feeding practices in all segments of the population. Comparison with data from the 1969 Survey will provide data for the assessment of changes in breast and bottle feeding over the last decade.

PHS-6040

1. Project Description:

Three questions on the attitudes about and social influences surrounding breast feeding practices have been submitted and are included in the current National Natality Survey. This survey, currently in progress, will follow up 6,000 births at approximately 3 months of age with a mailed questionnaire to a 1 in 500 sample of mothers and will include demographic and some medical information on each birth. Data will be analyzed to assess which factors are associated with breast feeding and to estimate the current status of breast feeding in the United States.

The National Natality Survey is being conducted in 1979-80, rather than 1978-79 as was expected. Data will be sent to NICHD by August, 1981. Analyses will begin at that time.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00312-01 EB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
National Natality Follow-Back Survey and National Fetal Mortality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Project Officer:	Carl A. Keller	Acting Chief	EB/EBRP/NICHD
P.I. :	Paul Placek		NCHS

COOPERATING UNITS (if any)
National Center for Health Statistics

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The National Natality Follow-Back Survey is a mailed survey to mothers, physicians and hospitals for a 1 in 500 sample of legitimate births in the U.S. during calendar 1980. The National Fetal Mortality Survey is a similar survey to a 1 in 4 sample of all stillbirths over 20 weeks gestation.

PHS-6040

1. Project Description:

The NICHD submitted a number of questions concerning drugs used during pregnancy to be included on the NNFS and the NFMS which is currently in the field. Data collection will be completed during FY 1981 and we will receive a copy of the data tape after it has been cleaned and edited. Analysis of these data will begin upon receipt of the tapes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00313-01 EB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Effects of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Project Officer:	Michele Forman	Epidemiologist	EB/EBRP/NICHD
P.I.	:	Lechaim Naggan	Professor	Ben Gurion Univ.
Others	:	Heinz Berendes	Director	EBRP/NICHD
		Gilliam Lewand-Hurdt	Professor	Ben Gurion Univ.
		Shimon Moses	Professor	Ben Gurion Univ.
		Howard Hoffman	Statistician	BB/EBRP/NICHD

COOPERATING UNITS (if any)
Epidemiology and Health Services Evaluation Unit, Center for Health Sciences, Ben Gurion University on the Negev, Beer'Sheva, Israel

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.40	.40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This contract is designed to study the simultaneous effects of various exposures to westernization, in this case to Israeli society, on changes in infant feeding patterns among a nomadic population experiencing sedentarization. The contractor was let persuant to an RFP which specified that the study has to be conducted in a population undergoing life-style transition and that the total population of women delivering newborns is accessible to be interviewed 24-72 hours after delivery. Analyses will be done in collaboration with NICHD to assess the effects of various exposures on infant feeding patterns.

PHS-6040
(Rev. 10-76)

1. Contract Description:

The objectives of this contract are:

1. To document the present trend of infant feeding practices (breast and bottle feeding) among the three groups of Negev Bedouins: the semi-nomad, the transitional, and the sedentary. This will be achieved by a cross sectional study, interviewing a stratified sample of 900 women who gave birth 5 to 8 months prior to the interview.

2. To describe in this cohort a set of characteristics distinguishing breast from bottle feeders in each of the three groups. To identify factors which influence women to choose to breast feed or bottle feed and determine whether these factors are indicative of exposure to "westernization."

3. To further use the information from this cohort of 900 women to describe whether Bedouin women have changed their infant feeding practices in the last few years, by getting information about older children, born earlier in calendar time to these same women.

4. To prospectively compare whether attitudinal statements of the Bedouin women after delivery, and past behavior (with regard to infant feeding practices of older children), correlate with their behavior 5 to 8 months later. This will be done by consecutive interviews of 1,500 mothers after delivery in the hospital and a second interview 5 to 8 months later.

5. To determine whether breast fed children in the prospective cohort have fewer hospital admissions and shorter average duration of hospital stay in the first 5 to 8 months of life regardless of category and other indices of westernization. This objective goes beyond the scope of the RFP but adds an important dimension of an outcome measurement of feeding practices.

NICHD ANNUAL REPORT

October 1, 1979 through September 30, 1980

Epidemiology Branch
Contract and Collaborative Research

Contract Title : The Epidemiology of Infant Mortality in Baltimore, MD

Contractor : Johns Hopkins University School of Public Health

Money Allocated: $30,114 (FY 1980)

Objectives: To elucidate the biological, social, and environmental variables which influence the high rate of infant mortality in the Baltimore metropolitan area.

Major Findings: Analyses of data have only recently begun; therefore, as of this date, there are no major findings to be reported.

Significance of Biomedical Research and Programs of the Institute: An important part of this study will be identification of factors which effect the infant mortality rate in an urban metropolitan area where infant mortality is often 15.2 times the national average. Identification of those factors may provide the opportunity to intervene on these variables and significantly reduce the infant mortality rate in such high risk populations.

Proposed Course: No additional funding is anticipated. Collaborative analysis, using NIH computing staff and facilities, is expected to continue for two years with interim publication of any significant findings.

Project Officer: Carl A. Keller, D.V.M., Ph.D.

NICHD ANNUAL REPORT

October 1, 1979 through September 30, 1980

Epidemiology Branch
Contract and Collaborative Research

Contract Title : The Relationship of Body Density To Other Methods of Assessing Obesity in Eight Year Old Children and The Association of Obesity With Present and Past Nutritional and Activity Factors and Parental Attitudes

Contractor : University of California, Berkeley School of Public Health

Money Allocated: NONE

Objectives: The objectives of this study are to develop a valid method for the measurement of body fat in 8 to 9 year old (pre-puberty) children. Practical suggestions for the use of anthropometric measurement of pre-pubescent obesity which can be accomplished with available clinical instruments will be determined. In addition, these measurements will be related to previous eating and activity practices as well as previous anthropometric measurements on the same children to assess the effects of early feeding and activity and the predictive values of early measures on pre-pubescent body fat content.

Major Findings: One of the preliminary findings of immediate interest is that the triceps skinfold thickness is most highly correlated with body fat determinations of all standard anthropometric measures in this young group of children, as is the core with post-pubescent children and adults.

Significance to Biomedical Research and Programs of the Institute: The results of this study should provide data for more accurate clinical measures of childhood obesity and early predictors for these measures.

Proposed Course: All data tapes have been received with documentation and are currently being analyzed. A final report on this subject will be completed during FY 1981.

Project Officer: Carl A. Keller, D.V.M., Ph.D.

NICHD ANNUAL REPORT

October 1, 1979 through September 30, 1980

Epidemiology Branch
Publications and Presentations

Publications:

Keller, C.A.: Epidemiological characteristics of pre-term births. In S. Friedman and M. Sigman (Eds.): Pre-term Births: Relevance to Optimal Psychological Development. Academic Press, New York, February, 1980.

Presentations:

Keller, C.A.: The Epidemiology of Prematurity. Presented at the United Nations at the Childbirth in America: The First National Conference, sponsored by The New York Hospital-Cornell Medical Center in Commemoration of The International Year of the Child, New York City, November 9, 1979.

Keller, C.A.: Are Prematurity and Neonatal Mortality Venereal Diseases? Presented at a seminar on Prematurity and Neonatal Mortality at Columbia University, New York City, November 30, 1979.

Forman, M.R. and Gussler, J.D.: A 24-Hour Recall of Infant Feeding. Pre sented at an informal seminar as a part of the 78th Annual Meeting of the American Anthropological Association, Cincinnati, Ohio, November 27-30, 1979.

Forman, M.F.: Breast and Bottle Feeding in the United States: An Illustration From the Pima Infant Feeding Study. Presented at a Maternal and Child Health seminar sponsored by the Department of Maternal and Child Health at The Johns Hopkins University School of Public Health, Baltimore, MD, March, 1980.

Keller, C.A.: The Epidemiology of Prematurity. Presented at the University of Maryland, College Park, MD, April, 1980.

Forman, M.F.: The Pima Infant Feeding Study: The Role of Socio-cultural Factors in the Trend in Breast and Bottle Feeding. Presented at the annual meeting of the American Pediatric Society, San Antonio, Texas, April, 1980. (Abstract)

NICHD Annual Report

October 1, 1979 through September 30, 1980

Biometry Branch

The Biometry Branch continued activities along three lines: (1) provision of statistical analysis and consultation to NICHD intramural and extramural investigators; (2) pursuit of individual and collaborative research in biometry and biostatistics; and (3) support of clinical trials initiated by the NICHD. While the Branch maintained its traditional strong ties to both the Intramural and Extramural programs of the Institute, this year international activities were also important.

In October of 1979, Mrs. Yupha Onthuam, Chief of the Data Processing Unit, Institute of Health Research, Chulalongkorn University, Bangkok, Thailand, came as a guest worker for one month's advanced study in biostatistics under the guidance of J. Schlesselman. In addition to completing a program of reading on selected topics in biostatistics, she finished work on two papers on drug addiction in Thailand, written in collaboration with Dr. Vichai Poshyachinda, Institute of Health Research.

In October of 1979, J. Schlesselman spent two weeks lecturing in biostatistics and working with faculty and students in the Department of Pharmacology at the Post-Graduate Institute of Medical Education and Research, Chandigarh, India. This assignment, at the invitation of the World Health Organization, was followed by a four day visit to the Institute of Health Research at Chulalongkorn University in Bangkok. A brief lecture series on clinical trials and case-control studies was presented, and statistical consultation given to medical investigators involved in metabolic studies of injectible contraceptives.

In December of 1979, at the invitation of the Department of Community Health, University of Trondheim, Norway, H. Hoffman spent six weeks as a Visiting Research Scientist. This invitation was sponsored by the Norwegian Council for Science and the Humanities. While in Norway, Mr. Hoffman gave seminars on recent research studies on perinatal mortality, consulted with members of the medical and engineering faculty on current research activities, and continued to analyze data from the Norwegian Medical Birth Registry in collaboration with Dr. Leiv Bakketeig. On the return from Norway, he presented a paper, on birthweight distributions and perinatal mortality within sibships, at a joint meeting of the British Society for Population Studies and the Royal Statistical Society (Medical Section).

In regard to work within the Epidemiology and Biometry Research Program, Biometry Branch staff have consulted with Dr. James Mills, advising on sample size and design for a collaborative study of malformations and fetal loss in pregnancies of women with juvenile onset diabetes mellitus. Branch staff are also involved in study organization, design of data collection forms, and data analysis. Advice has also been given on the design of a study of trends in breast feeding among Pima and Papago Indians over a 30 year period, under the direction of Dr. Michele Forman, and consultation has

occurred on two other proposed studies: (1) decision to breast feed by upperclass women, and (2) cultural changes and breast feeding in nomadic groups. Biometry Branch staff are active in a literature review of studies of teenage pregnancies and are involved in assessing the linked birth-death certificates from U.S. states relating to this issue.

A major committment of staff time and effort continued to support follow-up and analysis of a randomized clinical trial of the safety and efficacy of phototherapy in comparison with exchange transfusion for the treatment of hyperbilirubinemia. D. Bryla has devoted virtually all of her time to the analysis and data management of this project. The field phase, funded by contract with the Intramural Program and administered by the Scientific Director, NICHD, entered the sixth year of follow-up of children enrolled in the study. Since the sixth year examination is comprehensive, including physical, neurological, psychological and audiology data, a major effort has been devoted to the design of the necessary data collection instruments. The final editing of the one year data has been completed and analysis has been started.

A second clinical trial supported by the Branch concerns an investigation of the safety and efficacy of cysteamine in the treatment of nephropathic cystinosis. This study is being done in collaboration with Dr. Joseph D. Schulman, Human Biochemical and Developmental Genetics Section, NICHD, and with investigators at medical schools at the University of Southern California and the University of Michigan. The Biometry Branch will provide statistical analysis of the accumulating clinical trial data, with data processing support from E. Harley in EBRP.

Although the preceding two studies represented a major component of Biometry Branch support for intramural projects, a number of consultations were made during the past year, a summary of several being as follows.

Analysis of the partial separation of multiple forms of liver microsomal cytochrome P-450 was performed by E. Brittain in collaboration with Dr. Daniel W. Nebert and Dr. Matti A. Lang of the Developmental Pharmacology Branch, NICHD. The rates of formation of 17 P-450 mediated reconstituted monooxygenase activities were observed in each of 16 fractions. In order to determine if a difference existed between two activities, the profile of one activity over the 16 fractions was compared with the profile of the other. A component of the two factor analysis of variance, namely the test for interaction, was used to test for the lack of parallelism in the profiles. The results suggested that there are probably at least 15 different forms of P-450. An extension of this study is under way.

A statistical characterization of the natural history of nephropathic cystinosis is being performed by G.F. Reed in collaboration with Dr. Joseph D. Schulman, Human Biochemical and Developmental Genetics Section, NICHD. The data for this study are derived from the results of a randomized clinical trial of vitamin C, which tested and rejected the efficacy of ascorbic acid as a therapy for the disease. Given the ineffectiveness of the treatment, the pooled groups of the study subjects may be regarded as representative of the population of children afflicted at various stages in the course of the

disease. Accordingly, it is planned that statistical profiles at various ages will be constructed from the data. These profiles should prove valuable as a standard for management of cystinotic patients, as well as a reference for future trials.

An analysis of variance for determining the merits of a new assay for zinc measured in serum was performed in collaboration with Dr. James Hansen, Developmental Biology and Clinical Nutrition Section, NICHD.

A discriminant analysis of squirrel monkey vocalization data was completed for Dr. Harriet Smith, Laboratory of Developmental Neurobiology, NICHD. A manuscript for publication has been prepared.

Work with NICHD staff in the Extramural Program ranged from participation in ad hoc contract review committees, to site visits, to serving as co-project officer on NICHD contracts. Biometry Branch staff have worked closely with staff of the Center for Research for Mothers and Children (CRMC). H. Hoffman is serving as assistant project officer for the NICHD Cooperative Study of SIDS Risk Factors, funded by a contract with the Clinical Nutrition and Early Development Branch, NICHD. He is a member of the steering committee, has participated in site visits to all of the study centers and has been active in the sub-committees advising on initial analysis of data. He is also collaborating in reporting findings from a study of developmental neurophysiology and SIDS, funded by contract with CRMC.

J. Schlesselman has maintained close ties with staff in the Contraceptive Evaluation Branch and the Contraceptive Development Branch, CPR, serving on reviews of contract proposals and consulting with CPR staff on statistical issues relating to the design and analysis of clinical trials and case-control studies.

Staff of the Biometry Branch serve as consultants to outside investigators. H. Hoffman has consulted with Dr. Glenn Bartlett, Pediatric Outpatient Services, Hershey Medical School, Pennsylvania on indices of maturity at birth and the associated rates of perinatal mortality from the National Collaborative Perinatal Study. He has continued work with Dr. Josephine Weatherall, Medical Statistician, Registrar Generals Office, England and Mr. Peter Goldblatt of the Office of Population Censuses and Surveys, England on the analysis of British data on birth weight and gestational age in relation to major congenital malformations. He has continued collaboration with Dr. Don Gibson, National Institute of Aging, on the lifespan characteristics of mice and rats derived from NIA contract data, and has consulted with Dr. Thomas Wehr, Clinical Psychology Branch, NIMH on time series analysis of long-term measures (temperature, mood ratings, sleep amount, activity, etc.) in patients with manic-depressive illness. He has also been called upon to advise Dr. Bryan McCarthy, Center for Disease Control and the Georgia State Health Department, on a longitudinal study of consecutive births to white and black mothers in Georgia 1972-1976. He participated in a contract review, funded by the Division of Research Resources, NIH, of the BIOMED computer package and has continued as a consultant to the Institute of Laboratory Animal Resources, National Academy of Sciences.

J. Schlesselman is serving as a statistical advisor to Dr. Howard Ory, Center for Disease Control, on a case-control study of oral contraceptive use and breast, endometrial and ovarian cancer, funded under contract by the CPR, NICHD. He has presented a seminar, for Dr. Ory's staff, on the application of logistic regression and log linear models to the analysis of case-control data, and has reviewed contract proposals from organizations competing to serve as the data processing center for the study. As the study progresses he will become fully involved in the data analysis. He has also served as a reviewer of proposals for statistical support of the Brain Tumor Study Group, funded by the National Cancer Institute, and has participated in drafting a paper on "Weak Associations in Epidemiology", based on a workshop of the American Health Foundation.

Other activities of Biometry Branch staff include serving as referees for papers submitted to the Journal of the American Statistical Association, American Journal of Epidemiology, Biometrics, and Obstetrics and Gynecology.

The departures of some staff from the Biometry Branch have occassioned the recruitment of three persons in the past year: Ms. Dorothy Day (Branch Secretary), Ms. Erica Brittain (Mathematical Statistician) and Dr. George F. Reed (Mathematical Statistician).

A summary of publications, talks and research projects follows.

NICHD Annual Report

October 1, 1979 through September 30, 1980

Biometry Branch

Publications and Talks

Publications:

Bakketeig, L.S., Hoffman, H.J.: Perinatal mortality by birth order within cohorts based on sibship size. Brit. Med. J. 2: 693-696, 1979.

Bakketeig, L.S., Hoffman, H.J., Harley, E.E.: The tendency to repeat gestational age and birth weight in successive births. Amer. J. Obstet. Gynecol. 135: 1086-1103, 1979.

Bakketeig, L.S., Hoffman, H.J.: Pregnancy order and reproductive loss. Brit. Med. J. 1: 716, 1980.

Schlesselman, J.: How does one assess the risk of abnormalities from human in vitro fertilization? Am. J. Obstet. Gynecol. 134: 135-148, 1979.

Stadel, B.V., Sternthal, P.S., Schlesselman, J.J., Hall, D.W., and Ahluwahlia, B.: Variation of ethinyl estradiol blood levels among healthy women using oral contraceptives. Fertil. Steril. 33: 257-260, 1980.

Corash, L., Spielberg, S., Bartsocas, C., Boxer, L., Steinberg, R., Sheetz, M., Egan, M., Schlesselman, J., and Schulman, J.D.: Reduced chronic hemolysis during high dose vitamin E administration in Mediterranean type glucose-6-phosphate dehydrogenase deficiency. N. Engl. J. Med. (In press).

Dixon, G., Schlesselman, J.J., Ory, H.W., and Blye, R.P.: Ethynylestradiol and conjugated estrogens as postcoital contraceptives. J. Am. Med. Assoc. (In press).

Makinodan, T., Draper, H.H., Florini, J.F., Harrison, D., Hoffman, H.J., Holland, J.M., Hsu, C.K., Sprott, R.L.: Mice. In Cohen, B.J., Greenhouse, D.D. (Eds.): Animal Models for Research on Aging. Washington, D.C., National Academy of Science, pp. 17-82, 1980.

Bakketeig, L.S., Hoffman, H.J.: Interpreting Survey Data. In: Perinatal Audit and its Application to Clinical Practice. London, U.K., Royal College of Obstetricians and Gynaecologists. (In press).

Hoffman, H.J., Bakketeig, L.S.: Human Fetal Death Rates-- Relation to Indices of Maturity at Birth and Maternal Demographic Factors. In Hook, E.B., Porter, I.H. (Eds.): NYS Birth Defects Institute Symposium X-- Reproductive Loss-- Human Embryonic and Fetal Death. New York, N.Y., Academic Press. (In press).

Bakketeig, L.S., Hoffman, H.J.: Epidemiology of Preterm Birth: Results from a Longitudinal Study of Births in Norway. In Hendricks, C.H., Elder, M.G. (Eds.): Butterworths International Medical Reviews, Obstetrics and Gynaecology, Volume I - Preterm Labour. London, U.K., Butterworths and Co., Ltd. (In press).

Talks:

Bakketeig, L.S., Hoffman, H.J.: Perinatal mortality related to the tendency to repeat gestational age and birth weight. Seventh Nordic Perinatal Congress. Lillehammer, Norway, October 4, 1979.

Bakketeig, L.S., Hoffman, H.J.: Perinatal mortality related to maternal age and parity. A comparison of longitudinal and cross-sectional analysis. Seventh Nordic Perinatal Congress. Lillehammer, Norway, October 4, 1979.

Hoffman, H.J.: Risk for the sudden infant death syndrome as indicated by interrelationships among the state, EEG, respiration, apnea, and cardiac measures. American Academy of Pediatrics Annual Meeting. San Francisco, California, October 16, 1979.

Hoffman, H.J., Bakketeig, L.S.: Human fetal death rates: Birth weight, gestational age, maternal age, birth order and related factors. New York State Birth Defects Institue Symposium X on Reproductive Loss--Human Embryonic and Fetal Death. Albany, New York, October 29, 1979.

Hoffman, H.J.: Birth weight distributions and perinatal mortality within sibships. Joint meeting of the Royal Statistical Society (Medical Section) and the British Society for Population Studies. London, United Kingdom, January 11, 1980.

Schlesselman, J.J.: Lecture series in Biostatistics for the Clinical Pharmacology Training Program, Department of Pharmacology, Post-graduate Institute of Medical Education and Research, Chandigarh, India, October 25-31, 1979.

1. Design of multicenter drug trials
2. Adequacy of clinical trials for the evaluation of efficacy, and the analysis of follow-up data.
3. Application of case-control studies to monitoring adverse drug effects
4. Analysis of bleeding problems associated with IUD's
5. Sequential analysis
6. Concepts underlying tests of significance and confidence intervals
7. Methods of life-table analysis

Schlesselman, J.J.: (1) Design and analysis of randomized clinical trials. (2) Analysis of matched case-control studies. Institute of Health Research, Chulalonghorn University, Bangkok, Thailand, November 15, 1979.

Schlesselman, J.J.: Loglinear models and logistic regression in the analysis of case-control studies. Center for Disease Control, Atlanta, Georgia, May 21, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-850-04

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dolores A. Bryla	Statistician	BB	NICHD
Karen L. Fetterly	Computer Specialist	EBRP	NICHD
Nathaniel B. White	Math. Statistician	BB	NICHD
Leigh Baker (Summer)	Mathematical Aide	BB	NICHD

COOPERATING UNITS (if any) Downstate Medical Center, State Univ., N.Y.
Albert Einstein College of Medicine
Long Island Jewish-Hillside Medical Center
Medical College of Virginia
Univ. of Southern California Medical Center
Univ. of Cincinnati

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.50	1.25	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing phototherapy with non-phtotherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000 - 2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants 2,000 grams and above were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. Physical, neurological and mental development of these infants will be followed through six years of age.

PHS-6040

Objectives: Determine short and long-term benefits and risks of phototherapy for treatment of newborn hyperbilirubinemia.

Methods Employed: This study is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing treated with untreated infants under specific conditions. The Biometry Branch serves as a data center for this study, and is the focal point for receipt of examinations 1,339 newborns and approximately 1,000 follow-up examinations each year until the child is six years of age. These forms are checked for accuracy, and precoded in each center. The master files for each year's follow-up are edited for keypunch and coding errors, and for internal consistency. The Branch is also reponsible for the analysis of the data.

Major Findings: Since the clinical trial is continuing, final results are not yet available. An intensive analysis of the newborn data has been completed and the final versions of the manuscripts are being edited. Findings related to the newborn data will be published in a supplement to Pediatrics. The one-year follow-up data, which includes physical, neurological, ophthalmological, audiological and laboratory information, is being analyzed. The protocol, form-design and instructions for the sixth year follow-up examination were completed in the Spring of 1980. The examination will gather socio-economic, physical, neurological, audiological and psychological data.

Significance to Biomedical Research and the Program of the Institute: There is a well documented relation between hyperbilirubinemia during neonatal life and an increased risk of death or injury to the central nervous system from bilirubin encephalopathy, possibly resulting in impaired mental and neuromuscular abilities and deafness. Phototherapy has been widely accepted as a simple and effective treatment to reduce high bilirubin levels. In 1971, 51% of a sample of 300 U.S. hospitals were using phototherapy as a treatment procedure. In this study, phototherapy will be appraised for its immediate safety and efficacy, and for its possible long-term implications.

Proposed Course: To continue the analysis of the one-year follow-up data, with particular emphasis on the neurological, mental and physical data. Coding instructions will be developed for the six-year follow-up examination forms in the fall of 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 00 801 Z01-HD-~~00501~~-04

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies based on the Medical Birth Registry of Norway, 1967-1973

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Hoffman	Mathematical Statisticain	BB	NICHD
Other:	May Chiu	Statistician	BB	NICHD
	Ernest Harley	Supervisory Computer Spec.	EBRP	NICHD
	Karen Fetterly	Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)

Institute of Hygiene and Social Medicine, University of Bergen, Norway
Department of Community Medicine, Univ. Of Trondheim, Norway

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway, (2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers,(3) perinatal mortality in relation to order of birth and size of sibship, and (4) epidemiologic risk factors for preterm birth.

Methods Employed: Methods which have been used to analyze these data include life-table techniques, correlation and examination of bivariate distributions (based on contour diagrams), indirect standardization for rates, and other procedures applicable to cross-tabulations of data. The epidemiologic approach is that of a "prospective" population-based design which permits analyses by case-control, cross-sectional or longitudinal (cohort) means.

Major Findings: Several important findings have emerged from these studies. Norwegian births have been shown to be heavier and not as preterm as a comparable group of U.S. White births. A marked tendency for repeating low birth weight and/or preterm delivery has been demonstrated for successive pregnancies in this population of women. Recent studies have explored the relation of perinatal mortality to birth order and size of sibship. As sibship size increases, perinatal mortality rates also increase. However, conditional on size of sibship, perinatal mortality rates decline as birth rank in sibship increases. The rates of low birth weight delivery ($\leq$2,500 gms.) follows the same pattern generally as that of perinatal mortality. The incidence of preterm delivery (less than 36 weeks gestational age) also follows the same pattern. These associations may help account for the decline in perinatal mortality as birth rank increases.

The epidemiology of preterm birth has been the subject of a special inquiry. Many of the previously-known risk factors (such as low maternal age, unwed mother, lower social class status, occurrence of previous low birth weight or preterm birth, etc.) were also shown to exist in this data set. Placenta previa/abruptio or bleeding early in pregnancy (threatened abortion) were very strong risk factors for a preterm delivery. However, maternal age greater than 35, previous occurrence of a malformed birth, or toxemia in the current pregnancy were clearly not associated with the risk of a preterm birth. The present study was based on longitudinal data in which the first three consecutive single births to Norwegian mothers were analyzed. This approach is unique in the field of perinatal epidemiology, and is possible because of the special attributes of this data collection system.

Significance to Biomedical Research and the Program of the Institute: These studies have provided information on several issues pertinent to programs of the Institute. The "repeater" studies have helped to elucidate some of the etiologic problems of prematurity. Studies of fetal and infant mortality, and especially comparisons with available U.S. data sets, will aid understanding of the cause-specific mortality categories in which the U.S. rates may be higher than Scandinavian rates.

Proposed Course: About 15 studies based on the Norwegian Medical Birth Registry data have been published in association with Professor Bakketeig. Another five papers are currently in preparation. These studies are completing a collaboration which began while Dr. Bakketeig was a Visiting Scientist, EBRP, NICHD.

Publications:

1. Bakketeig, L.S., Hoffman, H.J.: Perinatal Mortality by Birth Order within Cohorts Based on Sibship Size. Brit. Med. J. 2:693-696, 1979.

2. Bakketeig, L.S., Hoffman, H.J., Harley, E.E.: The Tendency to Repeat Gestational Age and Birth Weight in Successive Births. Amer. J. Obstet. Gynecol. 135: 1086-1103, 1979.

3. Bakketeig, L.S., Hoffman, H.J.: Pregnancy Order and Reproductive Loss. Brit. Med. J. 1:716, 1980.

4. Bakketeig, L.S., Hoffman, H.J.: Interpreting Survey Data. In: Perinatal Audit and its Application to Clinical Practice. London, U.K., Royal College of Obstetricians and Gynaecologists, 1980. (in press)

5. Bakketeig, L.S., Hoffman, H.J.: Epidemiology of Preterm Birth: Results from a Longitudinal Study of Births in Norway. In Hendricks, C.H., Elder, M.G. (Eds.): Butterworths International Medical Reviews, Obstetrics and Gynaecology, Volume I - Preterm Labour. London, U.K., Butterworths & Co., Ltd., 1980. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-HD-00802-05

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study of Linked Information on Infant Death Certificates and Live Birth Certificates for Selected U.S. States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Hoffman	Mathematical Statistician	BB	NICHD
Other:	May Chiu	Statistician	BB	NICHD
	Ernest Harley	Supervisor Computer Sp.	EBRP	NICHD
	Karen Fetterly	Computer Sp.	EBRP	NICHD

COOPERATING UNITS (if any)

Departments of Health in the following states: California, Colorado, Minnesota, Missouri, New York, North Carolina, Rhode Island and Washington

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective is to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g. birthweight, gestational age, maternal age and race, parity, etc.). These studies will be compared with similar studies on a 1950 and 1960 cohort of U.S. births. Additional comparisions will be made to linked data from Canada (1971), Great Britain (1970) and Norway (1976-1973).

PHS-6040

Methods Employed: Since linked data from birth certificates and infant death certificates is not available from the National Center for Health Statistics, we have dealt directly with individual State Centers for Health Statistics. We have chosen states which have routinely linked this information and are willing to cooperate in this venture. The data is received on magnetic tape from the individual states and merged into a common format. The job of editing these tapes and merging the information into a common file is accomplished by the computer programmers within the Office of the Director, EBRP. When the study data sets have been edited and merged into a common file, the data will be analyzed using a variety of statistical techniques ranging from simple cross-tabulations to the making of sophisticated contour diagrams.

Major Findings: Perinatal mortality has been analyzed as a function of maternal age for birthweight-specific categories using 500-gram intervals. Maternal age has been stratified by single years through age 19, and by five year age groups thereafter. We have found that among white births, infants born to 19 year old mothers experience higher rates of "early neonatal mortality" (deaths ocurring in the first six days of life) compared to births of mothers aged 20-24 years, 25-29 years, 30-34 years for each 500-gram birhtweight interval. Similarly, 18, 17, 16 and 15 year old mothers have successively higher rates of early neonatal mortality for each 500-gram birthweight category. Since these mortality rates are birthweight-specific, it is unlikely that the known association of preterm birth with younger maternal age can account for these results. Additional studies comparing fetal and still-birth mortality rates by maternal age groups and birthweight-specific categories are underway.

Significance to Biomedical Research and the Program of the Institute: This study will provide a unique resource for the statistical and demographic study of infant mortality and some associated conditions (prematurity, teenage pregnancy, illegitimate births, etc.). Also, cause-specific infant mortality can be compared between the states participating in the study. The data will be available for the period 1968 through 1974 and will also permit an examination of time trends during this period.

Proposed Course: Data was obtained from Upstate New York in April, 1980 and from Missouri in August, 1980. Data from Minnesota, North Carolina and Missouri were presented in an invited talk for the Tenth Birth Defects Symposium in Albany, New York, October, 1979. Some related analyses were also presented at a joint meeting of the Royal Statistical Society (Medical Section) and the British Society for Population Studies in London in January, 1980. Some of these results will be published soon, and other papers using these data are in preparation.

Publications:

Hoffman, H.J., Bakketeig, L.S.: Human Fetal Death Rates--Relation to Indices of Maturity at Birth and Material Demographic Factors. In Hook, E.B., Porter, I.H. (Eds.): NYS Birth Defects Institute Symposium X--Reproductive Loss--Human Embryonic and Fetal Death. New York, N.Y., Academic Press, 1980. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-HD-00806-03

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Case-Control Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James Schlesselman	Chief	BB	NICHD
	Daniel Denman	Statistician	BB	NICHD
Other:	Paul D. Stolley	Professor	Univ. Penn Medical School	

COOPERATING UNITS (if any)

University of Pennsylvania Medical School, Department of Research Medicine

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A book on Case-control studies is being written which will detail the logic of the study design, provide explicit recommendations for its application, and describe a variety of methods for the analysis of case-control data. The book is planned as a reference for researchers and advanced students in medicine, public health, epidemiology, and statistics.

PHS-6040

Project Description and Progress to Date: The entire book has been drafted and is now in the final stages of rewriting and editing. Chapters dealing with the following topics have been written: Advantages and Disadvantages of Experimental and Observational Studies, Approaches to the Assessment of Risk, Planning and Conducting a Case-Control Study, Use of Matching, Sources of Study Bias, Planning the Size of the Study, Basic Statistical Methods of Analysis, Multivariate Methods of Analysis, and Approaches to the Interpretation of Study Findings.

Significance to Biomedical Research and Program of the Institute: Case-control studies are extensively used to determine relatively rare adverse effects of drug or environmental exposure. For example, most of the adverse effects associated with use of oral contraceptives have been investigated using the case-control approach. Other current examples concern the discovery of an association between the development of vaginal cancer in young women with a previous maternal exposure to diethylstilbestrol during pregnancy, and studies of a potential link between bladder cancers and saccharin exposure in humans.

Proposed Course: The completed manuscript will be submitted to Oxford University Press in the Fall of 1980, with publication expected in mid 1981. The book will appear as the first in a new series in biostatistics and epidemiology, edited by Dr. Abraham Lilienfeld of the John Hopkins University.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00810-02

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Costs of Case-Control Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James J. Schlesselman	Chief	BB	NICHD
Other:	Bruce V. Stadel	Medical Officer	CEB	NICHD
	Erica Brittain	Mathematical Statistician	BB	NICHD

COOPERATING UNITS (if any)

Contraceptive Evaluation Branch, NICHD

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.15	.05	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A financial review of five case-control studies funded by the NICHD is being conducted to identify the costs associated with the planning, conduct and analysis of case-control studies. Expenditures associated with data collection, travel, operating expenses, and salaries of professional staff have been summarized for three phases of each study (1) planning, (2) data collection, and (3) analysis.

PHS-6040

Method Employed: A systematic review of project protocols and financial reports, on file in the NICHD contracts Office, has been done for five recently funded case-control studies.

Major Findings: Analysis of data regarding five case-control studies yielded several conclusions: (1) Salaries comprise about 60% of expenditures, (2) the majority of study costs are incurred during the data collection phase, (3) optimal allocation of cases and controls is of minimal value, and (4) the typical cost per subject was $150-200 (in mid 1970's dollars).

Significance to Biomedical Research and the Program of the Institute: Identification of costs associated with the planning, conduct and analysis of case-control studies will assist in the assessment of costs likely to be incurred in future studies funded by the Institute, and will provide a benchmark for gauging expenses associated with NICHD supported projects.

Proposed Course: A manuscript describing the results of this inquiry has been completed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00811-01

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

National Collaborative Cysteamine Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Erica Brittain	Statistician	BB	NICHD
Ernest Harley	Computer Spec.	EBRP	NICHD
Maria Keyser	Computer Tech.	EBRP	NICHD
James Schlesselman	Chief	BB	NICHD
Joseph Schulman	Chief	HBDG	NICHD

COOPERATING UNITS (if any)

University of California, San Diego
School of Medicine

University of Michigan
Medical School

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is a clinical trial to determine the safety and efficacy of cysteamine in the treatment of nephropathic cystinosis, a metabolic disease which usually leads to end-stage renal disease before 10 years of age. All children enrolled in the trial will receive cysteamine. Control information will be provided by data collected on 30 patients who were randomized to placebo in a previous trial evaluating the efficacy of Vitamin C for the treatment of this disease. Approximately 60 children will eventually be enrolled in the current trial, which is anticipated to last about three years. Evaluation of the drug's effectiveness will be chiefly determined by the levels of serum creatinine values of the treated children as compared with those of the historical controls.

PHS-6040

Objective: Determine the benefits and risks associated with treating cystinotic children with cysteamine.

Method Employed: This study is a clinical trial designed to determine the safety and efficacy of cysteamine in treatment of cystinosis. The trial will compare children treated with cysteamine with data collected previously on untreated children who were enrolled in the Vitamin C study. The Biometry Branch is responsible for key-punching data from examinations of approximately 60 children. Data obtained in follow-up examinations scheduled to occur three times a year will also be keyed. The Biometry Branch is responsible for analysis of the data, with computer support being provided by the EBRP data processing unit.

Major Findings: The trial has recently begun. No analysis has taken place at this time.

Significance to Biomedical Research and the Program of the Institute: Despite symptomatic treatment, children with cystinosis typically develop end-stage renal disease by age 10. It is believed that cysteamine may be effective in arresting the development of the disease, since it has been shown to significantly lower the free cystine content in fibroblasts cultured from cystinotic patients. This study will assess the efficacy and safety of cysteamine.

Proposed Course: To key data into the computer as it becomes available. Updated summaries of the data collected will be provided on a regular basis. Analyses which address emergent issues will be performed at regular intervals at the time sufficient data accumulates.

NICHD Annual Report
October 1, 1979 through September 30, 1980

Biometry Branch
Contract and Collaborative Research

Contract Title: Outcome of Successive Pregnancies for Norwegian Women 1967-1976

Contractor: University of Trondheim, Trondheim, Norway

Money Allocated: $5,250 (August 1, 1980 through December 31, 1980)

Objectives: This contract relies upon a unique data base to study some statistical aspects of repeating premature or mature births, in a well-defined national cohort of mothers. This study will make a valuable contribution to research aimed at elucidating factors that predispose to pre-term or low birth-weight delivery. Infant mortality will be studied as it relates to prematurity and the tendency to repeat similar gestational age and birthweight in subsequent deliveried to the same mother.

Major Findings: Summary data tabulations have focused on causes of death as a function of parity and birth weight ($\leq$1500, 1501-2500,>2500 grams). Earlier findings have shown that perinatal mortality increases as size of sibship increases, and that for a given size of sibship, perinatal mortality declines as birth order increases. The birthweight-specific cause of death data have shown that several particular causes of death are elevated for the higher risk low-parity-and-large-sibship births. As an example, deaths due to RDS are more common in this risk group. Also, deaths as a result of congenital anomaly are increased in this risk group. Several other causes of death do not occur more frequently than expected in this risk group. Most of the deaths are associated with a low weight birth. Since birth weight is a strong, independent risk factor, all of the comparisons apply to birth weight specific categories.

Recent results of this contract have also shown a strong tendency for perinatal death to be associated with births which are "light-for-length" at delivery, regardless of the birth weight. Crown-heel length is collected in the Norwegian Medical Birth Registry Data along with gestional age and birthweight. These three indices of maturity are closely related. However, in this large and comprehensive data set, these three measures provide several ways to study intrauterine growth retardation and the associated perinatal mortality.

Significance to Biomedical Research and Program of the Institute: An important result of this study will be to identify specific causes of death which are implicated by various risk factors for perinatal death. In addition to the risk associated with low-parity-and-large-sibship births, this study will investigate the risks of repeated low birth weight and/or preterm delivery. This tendency to repeat premature delivery is one of the strongest predictive associations known from previous studies of the epidemiology of prematurity.

Proposed Course: This contract will be completed by December 31, 1980.

Project Officer: Howard Hoffman

NICHD ANNUAL REPORT

Laboratory of Developmental Neurobiology

October 1, 1979 to September 30, 1980

Development and maturation of the brain remains the focus of this laboratory and our studies range from complex social interactions in primates to developmental changes in gene expression in neuronal cell lines. The bulk of the activities in the laboratory, however, are concerned with cellular, membrane and molecular level phenomena concerned with expression and regulation of neuronal function.

Analysis of neurodifferentiation in mammalian central nervous system cell cultures has progressed along two lines. 1) Development of cerebral cortex cultures and catecholaminergic explants in conjunction with a number of different probes of specific cell types to provide a more detailed picture of neural development in vitro. 2) These preparations and methods are being used in long term studies of the perturbations of development produced by pharmacologic and functional means. Changes in gene transcription take place during neuronal differentiation and attempts are being made to isolate and clone, using recombinant DNA techniques, those genes turned on and turned off during this process. Cell biologic studies of receptor synthesis, membrane insertion and aggregation within the membrane indicate that the relationship of surface receptors to cytoplasmic structures is a critical step and that neurons can modulate this relationship in muscle cells via a diffusible factor.

Proteins and small peptides play an increasingly recognized role in nervous system function and a variety of biochemical methodologies are being applied to understanding the cellular mechanisms by which these molecules are synthesized, transported and secreted. Glycosylation plays an important role in the regulation of cleavage of large precursor molecules into multiple active peptides.

Nerve growth factor (NGF) and other growth and mitogenic factors provide attractive models for the study of regulatory mechanisms concerned with nervous system development. Phosphorylation of specific nuclear proteins and modulation of surface receptors have been demonstrated as possible critical elements in the mechanism of NGF action.

Developmental aspects of the melatonin rhythm generating system are under investigation as well as the relationship of the system to the circadian rhythms of the suprachiasmatic nucleus of the brain. Purification of pinial N-acetyltransferase has progressed and this purification will provide a powerful tool for further studies of regulatory mechanisms in this system.

Squirrel monkeys exhibit a highly developed group social structure and their vocal repertoire is concerned at least in part in mediating social interactions. One set of vocalization seems particularly involved in affiliative interactions. Individual squirrel monkeys exhibit considerable

specificity in their use of these vocalizations with respect to other individuals and to the behavior context. This system may represent a rather high information mode of vocal communication in this primate, and may provide a model for human linguistic exchanges.

I. Section on Neurobiology

We can now assess quantitatively a number of aspects of neurobiologic function in mammalian central nervous system (CNS) cultures. 1) Total neuronal surface membrane (tetanus toxin binding), 2) cholinergic neurons (cholinacetyltransferase, or CAT), 3) Inhibitory neurons (glutamic acid decarboxylase, plus aminobutyric acid uptake), 4) Glia (-alanine uptake), 5) Benzodiazepine (BZP) binding neurons, 6) BZP binding non-neuronal cells, 7) cell counts of total neurons or of autoradiographically identified inhibitory cells. Both phenobarbital and phenytoin have deleterious affects on development of cerebral cortical neurons in culture. BZP binding and CAT activity were most affected while -aminobutyric acid (GABA) containing cells were less so. Phenytoin stimulated -alanine uptake and non-neuronal BZP binding.

Pre- and postsynaptic mechanisms in CNS cultures can be dissected physiologically by computer assisted statistical analysis of intracellulary recorded synaptic responses. Alterations in Ca^{++} and Mg^{++} ions primarily affect the output of transmitter in this system. We have cultured catecholaminergic neurons from the locus coeruleus together with spinal cord (SC) cells and preliminary experiments indicate that physiologically functional interactions develop between the catecholaminergic cells and the postsynaptic SC cells.

In studies of synapse formation in the neuromuscular system, we have found that a factor or factors produced by neuronal cells, when added to muscle cells: 1) aggregates acetylcholine receptors (AChR), 2) decreases the lateral mobility of AChR, 3) promotes the attachment of AChR to the myotube cytoskeleton, and, 4) decreases the rate of degradation of AChR.

II. Section on Functional Neurochemistry

The section continues the study of peptides and proteins which are involved in intercellular communication (i.e. as neurotransmitters, modulators and trophic agents), and on the cellular mechanisms by which these molecules are synthesized, transported and secreted.

Having demonstrated the existence of common prohormones for vasopressin and oxytocin and their respective neurophysins in the rat hypothalamo-neurohypophysial system, work is now in progress to identify the other peptide pieces which are also cleaved from the prohormones. These other peptides cleaved from the precursor may have a biological function as well. The conversion of the prohormones can occur within the secretory granules. Techniques for the bulk isolation of purified secretory granules from bovine pituitary have been developed, in order to study the proteolytic enzymes involved in the conversion of the prohormone, and for experiments on the mechanism of secretion of peptides.

The study of ACTH, MSH and endorphin biosynthesis and secretion have been conducted in the toad neurointermediate lobe. Previously we have demonstrated that these peptides are synthesized from a common, glycoprotein precursor (Mol. wt. 32,000) and the glycosylation is important for stabilizing the molecule from non-specific proteolysis, and in the processing to correct post-translational cleavage products. Similar results have now been obtained from studies of the precursor in the mouse neurointermediate lobe. The turnover and regulation of release of the ACTH, MSH and endorphin peptides from the toad neurointermediate lobe have been studied. These experiments have led to the discovery that there are two pools of ACTH, MSH and endorphin related peptides in the toad neurointermediate lobe that differ quantitatively in their peptide content, (one pool contains MSH and ACTH but no endorphin, while the other contains all three peptides). The use of such heterogeneous pools of these peptides may be a mechanism by which selective release of different sets of peptides, synthesized from a common prohormone, can be accomplished. Work is now in progress to determine the cellular mechanisms underlying the selective synthesis and storage of the different hormones in the different pools.

Finally, the use of the Squid Giant axon as a model for studies on the structural-functional relationships of various axonal cytoskeletal proteins will continue in collaboration with Dr. I. Tasaki.

III. Section on Intermediary Metabolism

Investigators in this Section are engaged in studies on the mechanism of action and the biological role(s) of nerve growth factor. Our intent is to understand the involvement of nerve growth factor and other growth and maturation factors in the regulation and maintenance of neuronal metabolism and development. We have observed that two specific nuclear proteins contain increased amounts of radioactive phosphate after the administration of nerve growth factor either *in vivo* or *in vitro*. Although these proteins have not been completely characterized as yet, they are independent, non-histone proteins with molecular weights of about 30,000. Both proteins are chromatin-bound and both occur in the neurons of the ganglia. TPA is a tumor promoter that, at least temporarily, blocks the nerve growth factor-induced outgrowth of neurites from sympathetic ganglia *in vitro*. We have shown that this material will also prevent the nerve growth factor-induced increase in nuclear phosphorylation. Increases in the phosphorylation of nuclear proteins also occur in PC12 cells cloned from a rat pheochromacytomia, but in the PC12 only one of the two proteins increased in the sympathetic neurons exhibit increased phosphorylation.

An exciting observation has been the finding that PC12 responds mitogentically to epidermal growth factor. When cells are treated with nerve growth factor they lose most of their response to epidermal growth factor. There is a marked decrease in the specific, high-affinity receptors for epidermal growth factor in the Na^{++} treated cells. These data lead to the hypothesis that the way, or one of the ways, in which nerve growth factor stops the division of these cells is to repress the synthesis of the receptors for mitogens. We hope by exploiting these various systems

to uncover the basic mechanism(s) of nerve growth factor action and to provide a better understanding of the biological role(s) of nerve growth factor and other factors in normal and malignant tissues.

IV. Section on Neuroendocrinology

The Section on Neuroendocrinology continues to conduct multidisciplinary analyses of the melatonin rhythm generating system.

First, studies of the central basis of circadian rhythms have established that lesions of the suprachiasmatic nucleus in the primate brain, which have severe disorganizing effects on the malatonin rhythm, do not alter the well described rhythm in cortisol. During the past year, extensive mathematical and histological analyses have verified the conclusion that the suprachiasmatic nucleus acts in the monkey as it does in rodents as a central driving oscillator controlling the melatonin rhythm. Second, the development of the melatonin rhythm generating system has been studied. The development patterns of a number of components have been examined, including beta-adrenergic receptors, the adenylate cyclase regulatory protein, adenylate cyclase, melatonin, biopterin, and the adrenergic stimulation of taurine release. These developmental patterns form a basis for future studies directed at understanding the factors controlling the precise schedule of biochemical differentiation of this system. Third, investigation of the adrenergically-stimulated efflux of taurine from the pineal gland have indicated that the process is not dependent upon calcium, microtubule assembly, a taurine gradient, protein synthesis, or oxidative phosphorylation. In addition, efflux is not dependent upon influx of sodium or amino acids. The mechanism, which appears to be cyclic nucleotide dependent, is not clearly understood at this time, but may be closely linked to mechanisms involved in the adrenergic hyperpolarization of the pineal cell, as depolarization treatments block efflux. Fourth, purification of pineal N-acetyltransferase has been greatly improved by incorporation of disulfide exchange chromatography, which together with affinity chromatography and adsorption chromatography, have yielded highly purified preparations of this enzyme. The available evidence suggests the enzyme may exist in a macromolecular complex. These studies are providing a more complete molecular description of this enzyme. Partially purified preparations have been used to raise antibodies to the enzyme, which appear to react with enzyme molecules and activate them. Fifth, studies aimed at understanding the regulation of N-acetyltransferase have established that disulfide containing peptides inactivate N-acetyltransferase. The most potent is insulin, which has recently been found throughout the brain. These findings point to the possibility that insulin-related peptides might be involved in the intracellular regulation of N-acetyltransferase *via* disulfide exchange reactions.

V. Section on Brain and Behavior

Considerable time and effort during FY 80 has been devoted to the evolution of plans to transfer the program of the Section physically from the Bethesda campus to NIHAC in Poolesville, MD. A much better environment exists in

that rural setting to continue studies of social behavior, which is easily disrupted. Scientific cross-fertilization with the NIMH group already at NIHAC (Dr. Paul Maclean's laboratory) will be an important additional benefit from the relocation. This move is expected to be completed in early FY 81.

The research programs in neurophysiology, vocal analysis, and behavioral genetics have all made progress during FY 80. New findings have been most satisfying in the area of correlating vocal behavior with patterns of affiliation in groups of squirrel monkeys. This work has identified the vocal forms most frequently used in affiliation and has described a number of the functional properties of this signalling system. Among the more interesting results are: 1) the sex differences in this form of vocalizing (it is exclusively female); and 2) the exchanges between preferred partners even when physically separated.

The neurophysiology program has focused on widening the data base of specialized single cortical neurons which function as feature detectors in the natural language of squirrel monkeys.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00047-11 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Biochemical studies of neuronal and other cell types.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.A. Neale	Physiologist	LDN NICHD
	B.K. Schrier	Medical Officer	LDN NICHD
	P.G. Nelson	Chief, LDN, NICHD	LDN NICHD
OTHER:			
	S.C. Fitzgerald	Biologist	LDN NICHD
	D. Warren	Bio. Lab. Tech	LDN NICHD
	K.F. Swaiman	IPA	LDN NICHD
	P.K. Sher	Clinical Associate	LDN NICHD

COOPERATING UNITS (if any)
W.H.Habig, DBP BB; D. Gallagher, AP M; W. Oertel LCS M

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.0	1.6

CHECK APPROPRIATE BOX(ES) X

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this research is to investigate the biochemical characteristics of developing central nervous system (CNS) cells in culture, as discussed in previous reports. We have undertaken two approaches to this study: 1) We have designed a series of biochemical assays to distinguish various types of neuronal and non-neuronal cells in cultures from spinal cord and cerebral cortex of fetal animals, and, 2) we have employed these assays to assess the effects of chronic treatment of the cultures with neuroactive toxins and drugs, including anticonvulsants. The data collected thus far show that clinically-relevant doses of phenytoin and phenobarbital have toxic effects on developing neurons in culture. The effects of the two drugs on different cell types appear to be different. In addition, tetrodotoxin, which blocks neuronal sodium channels, appears to be selectively toxic for CNS neurons, but spares dorsal root gangion neurons.

Project Description:

Objectives: Two principal goals are being pursued: 1) to understand the nature and regulation of development in the nervous system, and, 2) to assess the potential toxicity resulting from the use of nervous system active drugs in very young children.

Methods Employed: We have developed additional assays to be used in conjunction with neurotransmitter-related enzyme assays described in previous reports. These new methods include: 1) the biochemical, pharmacologic, and radioautographic assessment of neuronal versus non-neuronal specificity of a) labeling of cultures with ^{125}I-tetanus toxin, b) high affinity uptake of ^{3}H-γ- aminobutryic acid (GABA), and c) high affinity uptake of ^{3}H-β alanine; 2) correlation of the above parameters with total neuronal cell number estimated by random counts using phase microscopy, and 3) binding of ^{3}H-diazepam, and blockade of that binding by neuron-specific and non-neuron-specific benzodiazepines.

Major Findings:

^{125}I-tetanus toxin binding as a neuronal marker in CNS culture systems. High affinity ^{3}H-GABA uptake in these systems was maximally blocked by diaminobutyric acid (DABA), an inhibitor of neuronal GABA uptake, and was blocked to a much lesser extent by β-alanine, a selective inhibitor of glial GABA uptake. High affinity ^{3}H-GABA uptake paralleled the activity of glutamic acid decarboxylase (GAD), the GABA-synthesizing enzyme; thus, both assays appeared to reflect GABAergic systems in these cultures. ^{3}H-β-alanine uptake was limited to non-neuronal cells in both spinal cord-dorsal root ganglion (SC-DRG) and cerebral cortical cultures.

In SC-DRG cell cultures, chronic treatment with phenobarbital at concentrations in the clinically-relevant range, produced a dose-dependent decrease in choline acetyltransferase (CAT) activity and in the number of large SC cells in the cultures. Another anticonvulsant drug, valproic acid, did not have measurable deleterious effects on the cultures.

In cerebral cortical cultures, phenobarbital produced a concentration-related decrease in total neuron number, CAT activity, and neuron-specific diazepam binding, with lesser effects on total protein, GABA uptake, tetanus toxin labeling, and GAD activity. Phenytoin appeared to be somewhat more toxic, and in addition, was associated with an increase in the uptake of ^{3}H-β-alanine, a specific indicator of non-neuronal cells, and with elevated non-neuronal ^{3}H-diazepam binding. Limited studies with valproic acid indicated a much lesser degree of neurotoxicity.

Significance to Biomedical Research and the Program of the Institute: These studies indicate that 1) neuronal cell cultures are effective systems for evaluating certain aspects of relative drug toxicity, and that 2) developing neurons are adversely affected by phenobarbital and phenytoin, compounds which are among neuroactive drugs administered to pregnant women, neonates and children.

Proposed Course: Further evaluation of the effects of clinically used drugs on developing cells in culture will involve: 1) additional studies on the nervous system cell types affected by phenobarbital, phenytoin, valproic acid, and tetrodotoxin (TTX), 2) expansion of these studies to include other neuroactive drugs, and 3) further efforts to dissect the mechanism of the developmental effect of TTX.

Publications:

1. Godfrey, E.W., Schrier, B.K., and Nelson, P.G.: Source and target cell specificities of a conditioned medium factor that increases choline acetyltransferase activity in cultured spinal cord cells. Develop. Biol., 77: 403-418, 1980.

2. Bergey, G.K., Swaiman, K., Fitzgerald, S.C., Schrier, B.K., and Nelson, P.G.: Adverse effects of phenobarbital on morphological and biochemical development of cultured fetal mouse spinal cord neurons. Annals Neurol., in press.

3. Schrier, B.K.: Nervous system cultures as toxicologic test systems. In: Mitchell, C.L. (ed.): Nervous System Toxicology, New York, Raven Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00048-06 LDN

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of transcriptional level control of neurobiologic and behavioral phenomena

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.K. Schrier	Medical Officer	LDN	NICHD
	L.D. Grouse	Research Associate	LDN	NICHD
OTHER:	C.H. Letendre	Staff Fellow	LDN	NICHD
	P.G. Nelson	Chief, LDN, NICHD	LDN	NICHD
	D. Warren	Bio. Lab. Tech.	LDN	NICHD
	D. VanPutten	Bio Aid	LDN	NICHD

COOPERATING UNITS (if any)

P. Ross-Riveros, Dept. of Biology, University of Chicago

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.95	1.05	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous studies using molecular hybridization of polysomal poly(A)-containing messenger RNAs of undifferentiated (S) and differentiated (P) mouse neuroblastoma cells with their complementary DNAs (cDNAs) had shown that P cells contained some messenger RNA sequences not present in S cells. After further refinement of the hybridizations, it became clear that there were sequences specific to both S cells and P cells. The remainder of the year was devoted to attempts to clone, using recombinant DNA techniques (IMUA #108), these differentiation-step-specific sequences.

Project Description:

Objectives: The immediate objective is to clone the cDNAs which are complementary to these differentiation-step-specific sequences. The cloned sequences will be used in a variety of ways to trace nervous system development and to dissect some of the regulatory steps which control that development. Our ultimate goal is to understand that developmental program sufficiently so that we may effectively treat persons in whom that program has been disturbed.

Methods Employed: The methods for DNA and cDNA preparation, hybridization, and recycling of cDNA probes have been described in previous reports. We tried several methods of making cDNA double-stranded using AMV reverse transcriptase with varying degrees of success. We finally adapted the use of the Klenow fragment A of E.Coli. DNA polymerase I to obtain 100% double-stranded cDNAs from S and P cells. These double-stranded cDNAs have been trimmed of single-stranded material by treatment with S_1 nuclease. Methods are now being tested for the application of poly(dC) tails to the 3' ends of these molecules using the enzyme terminal deoxynucleotidyl transferase. Transcription products made by wheat germ, ascites, and reticulocyte systems, are being analyzed by two-dimensional gel electrophoresis by published procedures.

Major Findings: When the homologous mRNA-cDNA hybridization results were analyzed quantitatively, it was found that mRNAs from both differentiation states contained about 8000 different sequences, of which about 100 sequences occurred in high abundance. Heterologous hybridizations and recycling of the cDNA probes revealed that 9.7% and 6.8% of the messages in P- and S-cells, respectively, were specific to those differentiation states. The P-cell specific sequences included approximately 3 high abundance and 320 low abundance sequences. The S-cell specific sequences included approximately 3 high abundance and 250 low abundance sequences. From the products of many syntheses of cDNA, we collected about 1 g each of total S-cell and total P-cell cDNAs of low specific radioactivity, which have been made double-stranded and will be used for cloning. Another several preparations of high specific radioactivity cDNAs from each differentiation step is being recycled in order to obtain radioactive probes with which differentiation-step-specific clones may be identified. Analysis, by two-dimensional electrophoresis, of *in vitro* transcription products from S and P cells' messenger RNAs have shown the appearance of at least two P-cell specific abundant polypeptides. This work is continuing.

Significance to Biomedical Research and the Program of the Institute: We are working toward a specific understanding of transcriptional controls during differentiation and development in the nervous system. We hope to map out critical developmental requirements and essential gene products for differentiation in the mammalian nervous sytem. We

believe that this knowledge will be important for the understanding of normal and abnormal CNS development. In addition, the S-cell specific sequences may aid us in understanding the processes of mitotic control in tumor cells.

Proposed Course: We will continue to work toward obtaining clones of the S-cell and P-cell specific sequences. When we have obtained these, the following uses of them are planned: (1) Examination of the genes for these sequences to determine their nucleotide sequence, presence of R loops, etc. (2) Use of these sequences to determine when and where they are expressed in the nervous system. (3) Use of these cDNAs to extract and purify quantities of the corresponding mRNAs for identification of transcription products. (4) Examination of the regulatory processes which control synthesis of these sequences.

Publications:

1. Grouse, L.D., Letendre, C.H., and Schrier, B.K.: Sequence complexity and frequency distribution of poly(A)-containing messenger RNA sequences from the glioma cell line C6. J. Neurochem., 33: 583-585, 1979.

2. Grouse, L.D., Schrier, B.K. and Nelson, P.G.: Effect of visual experience on gene expression during the development of stimulus specificity in cat brain. Exper. Neurol., 64: 354-364, 1979.

3. Grouse, L.D., Schrier, B.K., Letendre, C.H., and Nelson, P.G.: RNA sequence complexity in central nervous system development and plasticity. In: Hunt, R., Monroy, A., Moscona, A. (eds.): Current Topics in Developmental Biology. Academic Press, in press, 1980.

4. Grouse, L.D., Schrier, B.K., Letendre, C.H., Zubairi, M.Y., and Nelson, P.G.: Neuroblastoma differentiation involves both the disappearance of old and the appearance of new poly(A)$^+$ messenger RNA sequences in poly-ribosomes. J. Biol. Chem., 255: 3871-3877, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00053-12 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Information processing in the central auditory system of mammals and birds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN NICHD
	D. Symmes	Chief, Section on Brain and Behavior	LDN NICHD

COOPERATING UNITS (if any)
Department of Primate Behavior, Max Planck Institute, Munich, Germany

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Single unit recordings are made from neurons in the auditory system of squirrel monkey. Species-typical vocalizations previously tape recorded are used as stimuli in a series of investigations aimed at disclosing systematic parameters of neural coding of this model language. Current emphasis is on the detailed nature of acoustic features or vocal fragments which appear prepotent in driving cortical cells.

PHS-6040

Project Description:

Objectives: This project is concerned with utilizing neurophysiological and neuroanatomical techniques to study processing of complex sounds in the primate central auditory system. Special attention is being paid to sounds used in intra-specific communication, to determine which cures are used by the brain and the animal to distinguish among the various elements in the communication repertoire.

Methods Employed and Major Findings: Work during FY 80 has extended our data base with respect to highly specialized feature detector cells in auditory cortex. Although the accumulation of these data has been somewhat slower than initial results led us to expect, we have now studied a substantial number of neurons (about 35) which process natural sounds in a manner consistent with the feature detector hypothesis. Specifically, these cells all respond to brief sound fragments imbedded within natural vocalizations with vigorous, short latency excitatory responses. Such "trigger features" have been found in almost all the vocalizations we work with, and it appears that some features are common to several vocalizations in our standardized test sequence. This finding helps us arrive at a satisfactory explanation of the fact that most (probably all) cells are driven by more than one vocalization or call type typical of this species. The new concept, then, is that short sound elements (combined in different sequences) are the basic units of this model language, and that the call type lexicon provided by behavioral observations is at a higher level of complexity. Our studies do not reveal to date the neural locus of fragment addition (i.e., those brain mechanisms which encode meaning). Our expectation at present is that these higher processes exist in cell assembly behavior and may not be elucidated by single cell recording techniques.

Significance to Biomedical Research and the Program of the Institute: These studies represent a rigorous and technically sophisticated effort to explore the brain mechanisms involved in a highly evolved, functionally active model language. The results have significance for understanding the development of brain mechanisms mediating communicative behavior in the lower primates, and ultimately have application in the area of normal and abnormal language function in man.

Proposed Course: To examine in depth the properties of responsive primary auditory cortex units in order to test the hypothesis that some fraction of these units may be described as feature detectors for biologically relevant acoustic fragments. To develop new approaches to the study of neural correlates of complete acoustic messages.

Publications:

1. Newman, J.D. and Symmes, D.: Feature detection by single units in squirrel monkey auditory cortex. In: Grutzfeldt, O., Scheich, H, and Schneiner, C. (eds.): Hearing Mechanisms and Speech. Exp. Brain Res. Suppl. 2, Berlin: Springer Verlag, pp., 140-145, 1979.

2. Symmes, D., Alexander, G.E. and Newman, J.D.: Neural processing of vocalizations and artifical stimuli in the medial geniculate body of the squirrel monkey. Hearing Res., in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00054-06 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Structural and behavioral analysis of vocal communication in squirrel monkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN NICHD
	H. Smith	Staff Fellow	LDN NICHD
	D. Symmes	Chief, Section on Brain and Behavior	LDN NICHD

COOPERATING UNITS (if any)

Veterinary Resouces Branch, DRS

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with 1) obtaining a detailed description of vocal behavior of squirrel monkeys, including the frequency of occurrence of defined microstructural features in a variety of behavioral contexts; and 2) delineating the relative contribution of genetic and environmental factors to development of normal vocal behavior. Beth feral and laboratory reared monkeys are studied, and methods employed include spectrographic analysis, manipulation of social context, and formal behavioral training.

PHS-6040

Project Description:

Objectives: This project is concerned with 1) obtaining a detailed description of vocal behavior or squirrel monkeys, including the frequency of occurrence of defined microstructural features in a variety of behavioral contexts; 2) delineating the relative contribution of genetic and environment factors to development of normal vocal behavior in infant monkeys.

Methods Employed and Major Findings: Our first series of correlative studies utilizing monkeys from naturally formed wild troops have been completed. These data include an extensive description of the patterns of social interactions in such groups, comparison with unselected social groups in similar contexts, and an analysis of the vocal concommitants of affiliative behavior. The data were presented by Dr. Smith in an invited address at the International Congress of Primatology, Florence, Italy, in July 1980.

The methods involved have three interelated aspects: 1) Quantitative description of behavior utilizing a focal animal sampling technique which sequentially encodes all behavior by each member of the social group; 2) Recording of vocalizations so that all vocal-nonvocal interactions are recoverable. Attribution of vocalizations to specific monkeys is done by both visual verification and retrospective statistical processing which utilizes samples of calls from each animal to construct discriminant functions which are then applied to "unknowns"; 3) Sound spectrographic analysis of vocalizations with emphasis on microstructural details and their variability.

The major findings are:

1. Stable, robust affilitative bonds exist among selective pairs of monkeys in these groups, and involve female monkeys primarily.

2. One call type with several variant forms (chuck call) is used more frequently during affiliative behavior than at other times. Only females make chuck calls.

3. Females sharing bond status are consistently more vocal when interacting with preferred partners than when interacting with other monkeys.

4. Chuck sequences (defined as two or more calls within one second) are most commonly exchanges between preferred partners, even when the individuals are physically distant and not otherwise interacting.

5. Males affiliate to some degree in captive groups, and utilize different vocalizations during affiliation. Males vocalize more

than females in some contexts, use the same vocalizations in many contexts, and seldom engage in sequence calling with other monkeys of either sex.

These findings are significant in that they disclose female usage of a call group, closely related to affiliation, which has a rich acoustical structure with syllable sequencing and individuality. Vocal exchange along pair-bond lines between physically separated individuals is previously unreported. We find the prospect exciting that these current vocal forms (highly specified, short range, socially related) in a very ancient lower primate may have more bearing on the evolution of our language than the territorial and agonistic shrieks and screams which have been the basis of much prior speculation.

Significance to Biomedical Research and the Program of the Institute: Squirrel monkeys possess one of the most specialized and discrete systems of vocal communication below man. An account of even part of the semantic aspects of this system would provide valuable insight into the function of animal languages in general and provide an excellent model for the study of language disorders in man. Also, knowledge of the otogeny of squirrel monkey vocalizations can contribute to an understanding of normal and abnormal speech development.

Proposed Course: To continue studies of social behavior and vocalizations as outlined above, and to improve the environment for such studies by developing more natural, partially outdoor, warm weather habitats at the NIH Animal Farm, Poolesville. We also propose to continue study of heritability of focal characteristics, both by inhouse breeding and outside acquisition.

Publications:

1. Lieblich, A.K., Symmes, D., Newman, J.D. and Shapiro, M.: Development of the isolation peep in laboratory-bred squirrel monkeys. Anim. Behav. 28: 1-9, 1980.

2. Smith, H.J., Newman, J.D., Hoffman, H. and Fetterly, K.: Statistical discrimination between vocalizations of individual squirrel monkeys. Anim. Behav., 1980, in press.

3. Symees, D., Newman, J.D.,Talmage-Riggs, G. and Lieblich, A.K.: Individuality and stability of isolation peeps in squirrel monkeys. Anim. Behav., 27: 1142-1152, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00056-05 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Regulation of ACTH, Endorphin, and MSH synthesis and secretion.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y.P. Loh	Research Chemist	LDN	NICHD
OTHER:	H. Gainer	Chief, Section on Functional Neurochemistry	LDN	NICHD

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neurointermediate lobe of the toad, (Xenopus laevis) pituitary synthesizes and secretes ACTH, αMSH and βendorphin. The lobe is under inhibitory control by dopamine neurons from the hypothalamus. We have demonstrated the biosynthesis of a common prohormone to ACTH, α MSH and β-endorphin. The predominant form of the prohormone is a 32,000 molecular weight glycoprotein. Studies on the significance of glycosylation of the prohormone, using an inhibitor of glycosylation, tunicamycin, has revealed that the carbohydrate is important for protecting the prohormone from random proteolysis and directing the post-translational cleavage of the molecule so as to yield the correct peptide products. Studies on the turnover of newly synthesized ACTH, α MSH and β-endorphin peptides in the toad neurointermediate lobe revealed the existence of a fast turnover and a slow turnover (storage) pool of these hormones. These two pools are regulated differently in their secretion.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the regulation of biosynthesis, turnover and secretion of ACTH, αMSH and endorphin peptides in the neurointermediate lobe of the toad (Xenopus laevis).

Methods Employed: Acid-urea gel electrophoresis, high performance liquid chromatography (HPLC), radioimmunoassays (RIA) for ACTH, αMSH and β endorphin.

Major Findings: αMSH, ACTH, and βendorphin are synthesized in the toad neurointermediate lobe from a glycosylated prohormone. Pulse-chase studies on the intact lobe using the inhibitor of glycosylation, tunicamycin, and in vitro studies on the cleavage of glycosylated and non-glycosylated forms of the prohormone by trypsin, revealed that the carbohydrate is important for the processing of the prohormone to correct products. Similar results have also been observed for the prohormone in the mouse neurointermediate lobe. Long-term (10-16h) pulse-chase studies on the toad neurointermediate lobe, inhibited from release by dopamine, revealed the existence of two pools of ACTH, αMSH and endorphin related peptides. One pool consisting of ACTH, αMSH and βendorphin, was rapidly degraded, while the other pool consisting of ACTH, αMSH and βLPH (but no β endorphin) was stored when the lobe was inhibited from release. Both pools were inhibited from release by dopamine, but the storage pool wa also inhibited from release by L-isoproterenol.

Analysis of melanotropic peptides in the rat brain revealed an abundance of MSH and an αMSH-like peptide, which has been identified as des-acetyl α MSH. The ontogenesis of these peptides in the fetal rat pituitary and brain have been analysed. The level of immunoreactive αMSH-like peptides in brain increased at the time of parturition and continued to rise after birth.

Significance to Biomedical Research and the Program of the Institute:

Peptides such as αMSH and βendorphin have been implicated in higher brain functions (e.g. avoidance learning and analgesia, respectively). While MSH is clearly important for melanophore control in lower vertebrates, and more recently βendorphin has been found to potentiate this action, the function of these peptides in mammals are less clear. Studies on the biosynthesis and regulation of secretion of these peptides from the pituitary and brain may provide insights into their functional significance. Recently, αMSH has been implicated in fetal development and elevated levels of βendorphin has been detected during labor, suggesting a possible role for this peptide in the relief of the pain during childbirth.

Proposed Course: 1) To further determine the mechanisms (e.g. selective processing of the prohormone or selective degradation of the cleavage products) by which different peptides, presumably synthesized from a similar prohormone, are accumulated in the two hormone pools.

2) To study the proteolytic enzymes involved in the processing of the ACTH/αMSH-endorphin prohormone, and to determine the site of intracellular processing. 3) To examine the roles of the ACTH, α MSH and endorphin peptides in fetal development and neuronal differentiation (using tissue culture models as well).

Publications

1. Loh, Y.P. and Gainer, H.: The role of carbohydrate in the stabilization, processing, and packaging of the glycosylated ACTH-Endorphin common precursor in toad pituitaries. Endocrinology, 105: 474-487. 1979.

2. Loh, Y.P., Eskay, R.L. and Brownstein, M.: MSH-like peptides in rat brain: Identification and changes in level during develoment. Biochem. Biophys. Res. Comm. 94: 916-923, 1980.

3. Loh, Y.P. and Gainer, H.: In vitro evidence that glycosylation of pro-opiocortin and corticotropins influences their proteolysis by trypsin and blood. Molecular and Cellular Endocrinology, in press.

4. Loh, Y.P.: Processing, turnover and release of corticotropins, endorphins and melanotropin in the frog pituitary intermediate lobe. In: CIBA Symposium No. 81, on "The Intermediate Lobe of the Pituitary." Pitman Medical Ltr., in press.

5. Loh, Y.P., Jenks, B.G. and Broadwell, R.D.: The role of the carbohydrate in the stabilization, processing and packaging of pro-ACTH/ MSH-endorphin in frog and mouse pituitary intermediate lobes. In: Biosynthesis, modification and processing of cellular and Viral Polyproteins. Eds. G. Koch and D. Richter, Academic Press, New York, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 HD 00057-05 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Axonal proteins: Biosynthesis, transport, neuronal cytoskeleton, and glial-neuronal interactions.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Gainer Chief, Section on Functional Neurochemistry LDN NICHD

COOPERATING UNITS (if any)

I. Tasaki and J. Baumgold, Laboratory of Neurophysiology, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The use of model giant axons in the squid and crayfish has led to the formulation of the "Glial-Neuronal Protein Transfer Hypothesis." In addition, the giant axon has been a useful model for the study of 1) biochemical bases of excitability, 2) Intra-axonal Protein Phosphorylation, and 3) The regulation of axonal growth by Ca^{++}-activated proteases. The mechanisms underlying axonal transport have been studied using rat neurosecretory (hypothalamic) cells and rat sciatic nerve. These studies emphasize the diverse roles of cytoskeletal proteins (e.g., actin and tubulin) in fast vs. slow axonal transport. A novel method, using covalent labeling probes, to study axonal transport has been developed.

PHS-6040

Project Description:

Objectives: To study the biosynthetic origins of axoplasmic proteins, the mechanism and role of axonal transport and their functions in axonal excitability and transport.

Methods Employed: Protein biochemical techniques, autoradiography, and intraaxonal and membrane protein labeling techniques.

Major Findings: The transfer of macromolecules (i.e., proteins) between schwann cells and axons in the squid and crayfish has been unequivocally demonstrated. Covalent labeling techniques in combination with intraaxonal perfusion in the squid has revealed a sub-membrane structure (morphologically and biochemically) involved in excitability. The most recent findings have been an intra-axonal divalent cation regulated phosphorylation mechanism which selectively phorphorylates neurofilament proteins. This phosphyorylation is also regulated by excitation of the axon. In addition, we have found an intra-axonal Ca^{++} activated protease which selectively degrades neurofilament proteins, and have analysed its properties which are remarkably similar to Ca^{++}-activated proteases acting specifically on intermediate filament proteins in non-neuronal systems. Given the specificity of the proteases in each case, and yet their ability to recognize each others unique endogenous substrate, suggests a common structural conformation between all the diverse protein subunits which form intermediate filaments.

The fast transport of secretory granules by mammalian neurosecretory cells is accompanied by a co-transport of cytoskeletal proteins in a form unlike those found conveyed in slow transport. This suggests that the former proteins may be involved in the actual fast transport mechanism. We have recently developed a new approach to study retrograde axonal transport, and have uncovered a major endogenous protein which moves in the retrograde direction.

Significance of Biomedical Research and the Program of the Institute: This definitive demonstration that macromolecules can be transported from glia to neurons, represents the first example of such metabolic cooperation between cells in the nervous system, in situ. Such a mechanism may underly trophic interactions, and be a basis for inter-cellular communication during development. The Ca^{++}-activated proteases we have studied may play a major role in Wallerian degeneration, and formation of new nerve endings during regeneration and development.

Proposed Course: To study the significance of the neurofilament phosphorylation and degradation by the Ca^{++}-activated protease as basic mechanisms which regulate axonal growth and cytoskeletal turnover. To apply the new technique to study axonal transport (developed by Dr. Fink) to a wider variety of neuronal systems, in particular to axon systems in the brain.

Publications:

1. Pant, H.C., and Gainer, H.: Properties of a calcium-activated protease in squid axoplasm which selectively degrades neurofilament proteins. J. Neurobiology, 11: 1-12, 1980.

2. Pant, H.C., Pollard, H., Pappas, G.D. and Gainer, H. Phosphorylation of specific distinct proteins in synaptosomes and axons from squid nervous system. Proc. Natl. Acad. Sci. 76: 6071-6075, 1979.

3. Fink, D. and Gainer, H. The use of a labeled acylating probe for the study of fast axonal transport, in vivo. Brain Res. 177: 208-213, 1979.

4. Fink, D. and Gainer H. Retrograde axonal transport of endogenous proteins in sciatic nerve demonstrated by covalent labeling in vivo. Science 208: 303-305, 1980.

5. Fink, D. and Gainer, H. Axonal transport of Proteins-A new view using in vitro covalent labeling. J. Cell Biol. 85: 175-186, 1980.

6. Lasek, R.J. and Gainer, H.: Metabolic interactions between neurons and glia. Ann. Rev. Neuroscience, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00058-05 LDN

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biosynthesis and secretion of peptides in the vertebrate nervous system.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Gainer	Chief, Section on Functional Neurochemistry	LDN	NICHD
OTHERS:	J. T. Russell	Staff Fellow	LDN	NICHD

COOPERATING UNITS (if any)

M. Brownstein, Laboratory of Clinical Science, NIMH

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Functional Neurochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using the rat hypothalamo-neurohypophysial system as a model, we have isolated two large molecular weight common precursors (mol. wt. ~20,000) for vasopressin and its neurophysin (pro-pressophysin) and oxytocin and its neurophysin (pro-oxyphysin). Pro-pressophysin was shown to be glycosylated while pro-oxyphysin was not. A glycopeptide, presumably cleaved from pressophysin, since it is absent in rats with heredity diabetes insipidus, which do not synthesize vasopressin, has been isolated and its partial amino acid sequence determined. We have also demonstrated that the two prohormones are present in secretory granules, and their processing to the respective hormones can occur intragranularly.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) To determine whether the neurohypophysial peptide hormones, vasopressin and oxytocin and their respective neurophysins are synthesized from a common precursor, and to further characterize these precursors. 2) To study the cellular mechanisms involved in the biosynthesis and secretion (by exocytosis) of these peptides. This vertebate neurosecretory system is ideal as a model for the development of necessary techniques and approaches for the study of peptidergic neurons and systems in general. The latter represents the major long term objective of this section.

Methods Employed: Gel electrophoresis, high performance liquid chromatography, thin-layer chromatography, radioisotope technology, stereotoxic intracerebral micro-injection, and isolation of brain nuclei for analysis, affinity chromatography, radioimmunoassay of vasopressin, oxytocin and neurophysin, sub-cellular fractionation techniques, spectrophotometric and spectrofluorometric techniques for measuring membrane potential.

Major Findings: The prohormones for vasopressin and oxytocin were isolated and subjected to limited proteolysis by trypsin. The tryptic cleavage products from each of the prohormones were separated and identified as vasopressin and oxytocin and their respective neurophysins. Thus, we have demonstrated that vasopressin and its neurophysin and oxytocin and its neurophysin are each synthesized from a common prohormone. Subcellular fractionation studies revealed the presence of the two pro hormones in secretory granules and their processing can occur intra-granularly. We have also shown that the vasopressin prohormone is a glycoprotein, and have isolated two small glycopeptide products that were presumably cleaved from the vasopressin precursor, since these peptides were absent in rats with hereditary diabetis insipidus which lack vasopressin. One of these glycopeptides was isolated from rat posterior pituitaries in a pure form and its amino acid composition and partial sequence were determined.

A method for preparing a highly purified preparation of neurosecretory vesicles from bovine posterior pituitaries was developed. The pH of the interior of these secretory vesicles was determined to be 5.5 using radioactive methylanine distribution. Further, the vesicles were shown to have an internal negative membrane potential. ATP in the presence of Mg^{2+} caused a large depolarization of these vesicles, and the presence of permeable Cl^- ions caused them to lyse osmotically.

Significance to biomedical research and the program of the institute: The identificaton of another peptide product derived from the vasopressin prohormone raises the possibility that the hypothalamo-neurohypophysial neurons may synthesize and release other biologically active peptides besides oxytocin and vasopressin. Studies directed at the understanding of the cellular mechanisms underlying the prohormone processing, packaging, axonal transport and secretion of the peptides products synthesized by these neurons, in adult rats, are prerequisites for the subsequent analysis

of the regulatory and integrative roles these peptidergic neurons may play during development.

Proposed Course: To study the proteolytic enzymes, involved in the conversion of the oxytocin and vasopressin prophormones to the hormones. To analyse the chemistry of the secretory vesicle membrane and plasma membrane in order to understand the mechanisms underlying membrane interactions during the exocytosis (secretion) of these neurosecretory peptides.

Publications:

1. Russell, J.T., Brownstein, M.J. and Gainer, H.: Trypsin liberates an arginine vasopressin like peptide and neurophysin from a Mr 20,000 putative common precursor. Proc. Natl. Acad. Sci., 76: 6086-6090, 1979.

2. Gainer, H., Russell, J.T., and Fink, D.J.: The hypothalamo neurohypophysial system. A cell biological model for peptidergic neurons. In: Grotto, A.M., Jr., Peck, E.J., Jr., and Boyd, A.E., III, (eds.): Brain Peptides: A New Endocrinology, Elsevier, Holland, pp. 139-159, 1979.

3. Brownstein, M.J., Russell, J.T., and Gainer, H.: Synthesis, transport, and release of posterior pituitary hormones. Science, 207: 373-378, 1980.

4. Gainer, H. and Brownstein, M.J., Neuropeptides, In: Albers, R.W. and Siegel, G. (eds.): Basic Neurochemistry., Amer. Soc. Neurochem., in print.

5. Russell, J.T., Brownstein, M.J. and Gainer, H.: Biosynthesis of vasopressin, oxytocin and neurophysins; Isolation and characterization of the two common precursors (Propressophysin and prooxyphysin), Endocrin., 1980, in press.

6. Russell, J.T., Brownstein, M.J. and Gainer, H.: Time course of appearance and release of ^{35}S-Cysteine labelled neurophysins and peptides on the neurohypophysis. Brain Res., 1980, in press.

7. Russell, J.T., Brownstein, M.J. and Gainer, H.: ^{35}S-Cysteine labelled peptides transported to the neurohypophyses of adrenalectomized, lactating, and Brattleboro rats. Brain Res., 1980, in press.

8. Gainer, H., Loh, Y.P. and Russell, J.T.: Biosynthesis of neuronal peptides: implications for neurobiology. In: Heldman, E., (ed.): Neuroactive Compounds and Their Cell Receptors, Brazil, Krager Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HD 00062-04 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Brain mechanisms of vocal production in squirrel monkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN NICHD

COOPERATING UNITS (if any)
Laboratory of Brain, Evclution and Behavior, (Dr. Paul MacLean), NIMH
Department of Primate Behavior, Max Planck Institute for Psychiatry, Munich, Germany, Dr. Peter Mueller-Preuss

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vocalizations from adult squirrel monkeys with defined brain lesions are recorded onto magnetic tape and analyzed with the sound spectrograph. Subjects are tested and observed in a range of social contexts to characterize vocal changes following damage to specific brain structures.

PHS 6040

Project Description:

Objectives: This project is concerned with the brain sites and neural mechanisms by which vocalizations are initiated and controlled. A major objective is to form a link with our ongoing studies of the perceptual and behavioral aspects of vocal communication, leading to an overall understanding of the communicative process. A further goal is to correlate the brain mechanisms of vocalization with those controlling other forms of emotional behavior, to enlarge our understanding of the neural control of social behavior in general.

Methods Employed and Major Findings: Additional monkeys studied this year augment our analysis of tegmental lesions, and, together with earlier cases, permit a synthesis of our results on changes in Isolation Peep production. The experimental monkeys fall into 5 categories, according to the nature of their vocal defects: 1) reduction in rate of calling; 2) reduced intensity and shortened duration (these subjects also exhibit reduced calling rate); 3) addition of abnormal frequency components; 4) substitution of normal tonal structure with expiratory noise; 5) unstable frequency modulation ("infantile isolation calls"). Lesions correlated with the first two defects were either in the central tegmental tract (lateral to the periventricular gray) or in the caudal periventricular gray (caudal to the level of the nucleus of the oculomotor nerve). Lesions correlated with defects 3 and 4 were located moe rostralward in the periventricular gray. The fifth defect occurred in an animal with bilateral lesions of the lateral thalamic tegmentum and peripenduncular nucleus. Since defects 3-5 are pathological (not seen in normal adults) we surmise that the associated lesions disrupt neural circuitry involved in coordinating the motor act of uttering the Isolation Peep, rather than changing motivational level or tendency to vocalize. It is noteworthy that other vocalizations are unaffected in these experimental monkeys.

In related work a collaborative study with Dr. Mueller-Preuss demonstrated an anatomical and functional link between the anterior limbic cortex ("cingular vocalization area") and the auditory association cortex. Electrical stimulation of the cingulate region interrupted spontaneous activity and disrupted auditory responses of single units in auditory cortex.

This finding demonstrates that a brain region involved in controlling vocal production also modulates functional activity of another brain region involved in auditory perception.

Significance to Biomedical Research and the Program of the Institute: This project combines methods for the production and anatomical analysis of experimental brain damage, procedures for testing subjects in naturalistic contexts, and quantitative methods for

evaluating changes in vocal behavior. The significance of this combined effort is the promise it offers for discovering the specific brain structures controlling different aspects of vocal communication, including articulation, contextual analysis and emotional expression. This work is therefore relevant both to the pathology of social communication as well as to the neural mechanisms controlling emotional expression.

The discovery of a functional link between a brain region mediating sound production and another region mediating sound perception is the first such demonstration in a primate. This linkage may be of general relevance to the neural mechanisms controlling social communication in primate groups.

Proposed Course: We propose in the lesion studies to focus on more rostral components of the vocalization pathway, in particular the cingulate gyrus and related limbic structures. A wider range of contexts will be studied to enlarge the classes of vocalizations available for analysis following experimental brain damage.

Publications:

1. Mueller-Preuss, P., Newman, J.D., and Juergens, U. Anatomical and physiological evidence for a relationship between the "cingular" vocalization area and the auditory cortex in the squirrel monkey. Brain Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00064-04 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Neurobiologic studies of neurons and glia from the mammalian central nervous system in cell cultures.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.G. Nelson	Chief, LDN, NICHD	LDN NICHD
	E.A. Neale	Research Physiologist	LDN NICHD
OTHERS:	K. Marshall	Guest Worker	U. of Ottawa
	R. Pun	Visiting Fellow	LDN NICHD
	W. Ofrtel		LCS M
	W. Habig		DBP BB
	W. Hendelman		U. of Ottawa
	S. Fitzgerald	Biologist	LDN NICHD
	L. Bowers	Bio. Lab. Tech.	LDN NICHD

COOPERATING UNITS (if any)
Departments of Anatomy and Physiology, University of Ottawa

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed and utilized statistical methods and computer analysis of synaptic interactions between pairs of spinal cord (SC) cells and between dorsal root ganglion (DRG) cells and SC cells to study pre- and post-synaptic mechanisms in mammalian CNS cell cultures. Alterations in Ca^{++} and Mg^{++} ions primarily affect the output of transmitter in this system although minor post-synaptic affects may also occur.

Catecholaminergic neurons from the locus coeruleus have been co-cultured with spinal cord cells. Preliminary experiments indicate that physiologically functional interactions develop between the catecholaminergic cells and post-synaptic SC cells.

Radioautography of SC-DRG and cerebral cortical cultures indicates that ^{125}I-tetanus toxin and ^{3}H-GABA labeling are primarily neuronal, whereas ^{3}H-β-ananine labeling is primarily non-neuronal.

Project Description:

Objectives: To understand the mechanisms underlying mammalian central synaptic action and to study the factors affecting formation and development of central neurons and their synapses. Catecholaminergic and peptidergic mechanisms are particular areas of interest.

Method Employed: As in previous reports, we prepare cell cultures of mouse or rat cerebral cortex or mouse spinal cord (SC) plus dorsal root ganglia (DRG). Intracellular recordings are done in combination with a variety of methods for applying pharmacological agents and media with altered ionic composition. Tissue from the brain stem (including the locus coeruleus) is explanted in conjunction with SC cell cultures. Fluorescent microscopic techniques visualize catecholamine containing fibers. Light and electron microscopy are utilized to monitor morphological development, and to characterize cell type specificity and synaptic interrelationships of neurons in cultures prepared from a variety of central nervous system structures.

Immunocytochemical methods are used to demonstrate those cells containing specific neuropeptides or neurotransmitter-related enzymes, and radioautography, to label those cells with specific high affinity receptor and transport systems.

Major Findings:

1. Blockade of electrical activity in cerebral cortical cell cultures produces developmental deficits similar to those documented earlier in DRG-SC cell cultures.

2. The quantal content of DRG-SC synaptic responses is substantially less than that of SC-SC responses. Both can be analyzed in terms of the statistical variations in synaptic potential amplitude. The quantal content is a function of the divalent ion (Ca^{++} and Mg^{++}) concentration bathing the neurons, with transmitter release being facillitated by a high Ca^{++}:Mg^{++} ratio. Complex paterns of sensitivity to the frequency with which a synapse is activated have been noted.

3. Application of opiate peptides had no effect on calcium action currents in cultured mouse SC or DRG cells. Preliminary experiments indicate that, as we have shown earlier with the opiate etorphin, opiate peptides reduce transmitter release from DRG cells. This suggests that receptors for the opioids may be localized at transmitter release sites at neurite endings, rather than on the neuronal cell body.

4. Explant cultures of locus coeruleus survive and develop luxuriant process outgrowth which exhibits fluorescence characteristics of catecholamines. These processes establish dense contacts with

spinal cord neurons in co-cultures of locus coeruleus with spinal cord cell cultures. Initial experiments indicate that comparable responses in spinal cord cells can be obtained by application of nor-epinephrine and electrical stimulation of the locus coeruleus explants.

Both biochemical and radioautographic studies have shown tetanus toxin labeling to be primarily neuronal, with higher silver grain densities associated with neuronal processes than with somata. During a chase period after toxin incubation, there is a shift in the pattern of labeling consistent with a movement of toxin into synaptic boutons. There appears to be no massive block of γ-animobutyric acid (GABA) uptake or release associated with tetanus toxin induced paroxysmal excitatory neuronal activity.

Whereas ^{125}I-tetanus toxin appears to label all neurons, in radio-autographic experiments only a small percentage (10-15%) of neurons in SC-DRG and cerebral cortical cultures are heavily labeled by high affinity ^{3}H-GABA uptake. Non-neuronal cells are also labeled with ^{3}H-GABA but to a lesser extent than neurons. High affinity ^{3}H-β-alanine uptake labels non-neuronal cells in a pattern similar to ^{3}H-GABA, but does not label neurons.

Significance to Biomedical Research and the Program of the Institute: Developmental studies of the nervous system require powerful probes of neuronal function and an understanding of synaptic mechanisms. Catecholamine containing systems may well play a critical role in development of the brain and studies incorporating such systems are therefore important. Continuing studies on the relevance of functional activation and of peptides and catecholamines for the development of the brain should contribute to one of the central concerns of the Institute.

Proposed Course: We will utilize the electrophysioligic and computer technologies we have developed in further studies of synaptic mechanisms with particular attention to pre and postsynaptic action of catecholamines and enkephalin. Further experiments are needed with the locus coeruleus - spinal cord cocultures with both anatomical and electrophysiological methods. Co-cultures of locus coeruleus and cerebral cortical cell cultures are planned. Further analysis of the mechanism by which decreased electrical activity produces abnormal neural development are in progress.

Histochemical methods are being developed and adapted to culture systems to enable the visualization of both peptide and neurotransmitter-related markers within the same neuron. Immunohistochemical techniques in electron microscopy will be pursued in order to define the structural characteristics and synaptic relationships of peptidergic neurons.

The kinetics of tetanus toxin binding to cells in culture will be further investigated in order to maximize neuronal receptor-mediated binding. Attempts will be made to monitor the movements of the bound toxin in relation to electrophysiologic effects and transmitter uptake or release.

The course of development of nervous tissue cells in culture will be monitored using morphologic and biochemical probes. Anticonvulsant-treated cultures will be examined by electron microscopy, with particular emphasis on synapse development.

Publications:

1. Nelson, P.G. and Lieberman, M. (eds.): Excitable Cells in Tissue Culture, New York, Plenum Press, in press.

2. Nelson, P.G., Neale, E.A. and Macdonald R.L.: Electrophysiological and structural studies of neurons in dissociated cell cultures of the central nervous system. In: Nelson, P.G. and Lieberman, M. (eds).: Excitable Cells in Tissue Culture, New York, Plenum Press, in press.

3. Macdonald, R.L. and Barker, J.: Neuropharmacology of spinal cord neurons in primary dissociated cell cultures. In: Nelson, P.G. and Lieberman, M. (eds.): Excitable Cells in Tissue Culture, New York, Plenum Press, in press.

4. Nelson, P.G. and Bergey, G.K.: Pharmacological and developmental studies on mammalian central neurons in cell culture. In: Giacobini, E., Vernadakia, A. and Shahar, A. (eds.): Tissue Culture in Neurobiology, New York, Raven Press, 1980, pp 221-227.

5. Bergey, G.K., Fitzgerald, S.C., Schrier, B.K. and Nelson, P.G.: Neuronal maturation in mammalian cell culture is dependent on spontaneous electrical activity. Brain Res., in press.

6. Bergey, G.K., Martin, M.R. and Hermes, M.: Effects of D,L-α aminoadipate on postsynaptic amino acid responses of cultures mouse spinal cord neurons. Brain Res., 193: 199-208, 1980.

7. Marshall, K.C., Engberg, I. and Nelson, P.G.: Studies of EPSP mechanisms in spinal neurons. In: Krnjevic, D. (ed.): The Physiology of the Ia excitatory post-synaptic potential, in press.

8. Nelson, P.G. and Henkart, M.P.: Oscillatory membrane potential changes in cells of mesenchymal origin: The role of an intracellular calcium regulating system. J. Exp. Biol., 81: 49-61, 1979.

9. Nelson, P.G., Neale, E.A., Matthew, E., and Zimmerman, E.A.: A presynaptic locus of action for the opiates. In: Barker, J.L. and Smith, T.O. (Eds.): The Role of Peptides in Neuronal Function. New York, Marcel Dekker, Inc., in press.

10. Neale, E.A., Macdonald, R.L., and Nelson, P.G.: Neurons in culture are developmental models. JAMA, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00089-06 LDN

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Pineal-pituitary interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN NICHD
OTHER:	L. Tamarkin	Guest Worker	LDN NICHD
	S.M. Reppert	Clinical Associate	NPMB NICHD

COOPERATING UNITS (if any)
J. Martin, Ph.D., Dept. of Pharmacology, Washington Univ. Med. School; K. Kirk, LC, NIAMMD; Kevin Catt, Reproductive Biology Branch, NICHD; Mark Perlow, NIMH.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many life processes are regulated by the pituitary gland. This project studies how the pineal gland, influences pituitary function. The primary control of the pituitary gland is via the release of brain hormones. One of these hormones, luteinizing hormone releasing factor, regulates the release of a pituitary hormone necessary for normal reproduction, luteinizing hormone. A pineal secretion, melatonin, prevents the brain hormone from stimulating the release of luteinizing hormone. Similar effects are found using new synthetic derivatives of melatonin. The function of the pineal gland is drastically altered by environmental light and by drugs; pineal function also changes markedly during development. Thus it seems possible that as a result of these changes, and subsequent pineal-pituitary interactions, there will be significant changes in pituitary gland function resulting in turn in alterations in general health. Such an interaction may explain why the pineal gland can stop reproduction in certain animals when they are deprived of light. A second interaction of the pineal gland and the pituitary is through the pituitary adrenal axis. Studies have been performed to determine whether or not such a relationship exists in the rhesus monkey.

PHS-6040

Project Description:

Objectives: To chemically and physiologically characterize pineal compounds which influence function of the pituitary gland, including those aspects concerned with reproduction and adrenal function. To describe the mechanism through which pineal compounds act and to design drugs which are more potent and long lived as compared to the parent compound, or block the effects of the pineal compounds.

Methods Employed: A pituitary organ culture system and a pituitary cell culture system using neonatal and adult pituitary tissue are the model systems studied. This approach provides a highly sensitive and precise means of measuring the effects of pineal hormones on the pituitary gland. In vivo systems are also used to further explore the effects of compounds of interest and of alterations in pineal function. Immunoassay procedures are used to measure the amount of pituitary hormones released following treatment with test compounds, or under physiological conditions. The number of receptors present on the pituitary gland and on pituitary target tissues is determined using radioreceptor assays.

Major Findings: (1) It has been possible to synthesize a new derivative of melatonin, 6-methoxymelatonin. This compound was designed to prevent inactivation through hydroxyation at the six position. It has been found that the metabolism of this compound is slower than that seen with melatonin. (2) 6-Methoxymelatonin has been found to mimic the effects of melatonin in vitro, to block the effects of LHRH on LH release from the pituitary gland. (3) The effects of changes in photoperiod on the number of gonadotrophin receptors in the testis have been examined. It has been found that during a 24 hour period there is no change in the apparent number of receptors present in this tissue. (4) It has been discovered that shifts in the environmental lighting schedule from L:D 14:10 to a lighting schedule of L:D 10:14 results in distinct changes in the numbers of FSH, LH, and prolactin receptors in the testes, which raises the possibility that the pineal gland influences reproduction through mechanisms which alter the number of receptors for pituitary hormones that are present in the testis.

Significance to Biomedical Research and the Program of the Institute: These studies are consistent with the mission of the Institute to support research in reproductive biology. The results of these studies may lead to an increased understanding of the role of the pineal gland in reproduction and to the development of new drugs to alter reproduction by either mimicking the effects of pineal compounds or blocking their effect. One consequence of these studies is that abnormalities in reproductive physiology may be explained on the basis of pineal function. Finally, these studies may uncover a interaction between pineal and the adrenal gland.

Proposed Course of Project: (1) The relationship of pineal secretion of melatonin and the adrenal secretion of steroids will be studied under a variety of enviornmental conditions and as a function of shifts and photoperiods to determine if these two parameters are closely linked. (2) The

changes in testis receptors for pituitary hormones will be analyzed in depth, with one goal being the determination of the relative rate of change in receptor number seen during testicular regression and recrudesence. (3) The effects of melatonin on prolactin secretion will be studied.

Publications:

1. Martin, J.E., McKellar, S., and Klein, D.C.: Melatonin inhibition of the in vivo pituitary response to luteinizing hormone-releasing hormone in the neonatal rat. Neuroendocrinology 31, 13, 1980.

2. Martin, J.E., Kirk, K., and Klein, D.C.: Relative effects of 6-hydroxymelatonin and 6-difluromelatonin on LH-RH-stimulated release of LH. Endocrinology, 106, 398-401, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00093-06 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Mechanism of Action of Nerve Growth Factor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Guroff	Chief, Sec. on Intermediary Metabolism	LDN NICHD
OTHERS:	S. Vinores	Staff Fellow	LDN NICHD
	G. Dickens	Bio Lab Tech	LDN NICHD
	N. Tolson	Biologist	LDN NICHD
	D. End	Guest Worker	LDN NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Intermediary Metabolism

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this research is to provide information on the mode of action of nerve growth factor. Nerve growth factor is required for the development and maintenance of the sympathetic nervous system and may be prototypic for factors involved in differentiation or maturation of various cell types. The mechanism of action of nerve growth factor is of interest because it should lead to an understanding of the way in which gene expression is regulated in neurons and how such factors induce the synthesis of specific enzymes for the biosynthesis of the neurotransmitter, norepinephrine. In turn, such information may lead to an understanding of the development and differentiation of the non-dividing, non-replaceable neurons of the central nervous system, and to information about the tumors that arise from them.

Objectives: (1) To investigate the mechanism of action of nerve growth factor on the differentiation and maintenance of the sympathetic nervous system; (2) to delineate the intracellular events mediating the action of nerve growth factor in sympathetic and sensory neurons; (3) to detect and characterize other factors which direct the development of other types of neurons; (4) to study the interactions of nerve growth factor with other factors impinging on the cell, especially the mitogens; (5) to investigate the effects of nerve growth factor and its antiserum on the growth rate and characteristics of tumors of neural crest origin; (6) to characterize the action of nerve growth factor or other factors on the central nervous system; (7) to measure the levels of nerve growth factor and of nerve growth factor receptors in clinically relevant conditions, such as those involving tumors of the sympathetic nervous system or of other neural crest-derived tissues.

Methods employed: The methods include standard techniques of cell culture and in vivo investigations of the sympathetic nervous system. Enzyme assay, small animal surgery, and stereotaxic administration are used, as well as biological and immunological methods by which nerve growth factor is measured. Radioisotope techniques are employed with heavy emphasis of the use of protein labeling with ^{125}I and phosphorylation studies with ^{32}P. Slab gel electrophoresis provides an integral tool for the separation of nuclear proteins. Standard immunological techniques are employed for the preparation and use of the relevant antibodies.

Major findings: (1) The administration of nerve growth factor causes the phosphorylation of two specific chromatin-bound nuclear proteins in the neurons of the sympathetic ganglia; one of these proteins also responds in the nerve growth factor-sensitive clone, PC12; (2) PC12 cells respond to epidermal growth factor, a potent mitogen, and terminal differentiation of PC12 cells by nerve growth factor leads to a loss of receptors for epidermal growth factor; (3) the addition of nerve growth factor rapidly alters the adhesiveness of F98 cells, nerve growth factor receptors are present on these cells; (4) some of the effects in the central nervous system previously thought due to nerve growth factor are, in fact, due to renin, a frequent contaminant of nerve growth factor preparations; (5) TPA, a tumor promoter, intensifies the actions of nerve growth factor and of epidermal growth factor on PC12 cells; the combination of epidermal growth factor and TPA leads to differentiation.

Significance to Biomedical Research and the Program of the Institute: Nerve growth factor is the best characterized and, indeed, the first known growth factor. It is required for the growth and maintenance of sympathetic neurons. Its mechanism of action, therefore, is of extreme interest and only the outlines are apparent. A full understanding of the mechanism of action of nerve growth factor might lead to a better understanding of the mode of regulation of the growth of neurons and of their differentiation. Further, understanding of the mechanism by which nerve growth factor affects cell development may give clues as to the normal regulation of the growth of mammalian cells and, specifically, of neurons, and to the process of genetic expression. Then, studies on nerve growth factor may indicate ways in which the selectivity of

neural connectivity can be understood and, indeed, altered. A molecular understanding of nerve growth factor action may lead to a common thread for the action of growth and maturation factors in general on their target cells. Finally, an understanding of genetic expression and growth regulation in normal cells and in the tumors which arise from them may give some new insights into tumor cell biology.

Proposed course of the project: (1) Continued study of the nuclear events following nerve growth factor action on the cell; identification and characterization of the proteins containing increased amount of phosphate; identification of the sites of phosphorylation; effect of TPA and retinoids on the phosphorylation; attempts to duplicate this effect of nerve growth factor using purified nuclei. (2) Identification of the protein kinase responsible for the nerve growth factor-stimulated phosphorylation. (3) Use of PC12 to fully characterize the action of epidermal growth factor on these cells; determination of the mechanism by which nerve growth factor decreases the epidermal growth factor receptors; search for a similar relationship between nerve growth factor and mitogens in normal embryonic sympathetic neurons. (4) Description of the effect of nerve growth factor on the adhesiveness of F98; characterization of the nerve growth factor receptor on F98; detailed study of the effects of nerve growth factor and its antiserum on this tumor in vivo and in vitro. (5) Study of the effects of tumor promoters on the actions of nerve growth factor and epidermal growth factor; exploration of the morphological changes induced by the combination of epidermal growth factor and TPA. (6) Evaluation of the increases in neuronal markers in response to nerve growth factor; elucidation of the nerve growth factor-induced increase in neuron specific enolase in PC12 cells and in normal adrenal medullary cells. (7) Attempt to measure nerve growth factor receptors in human tissue.

Publications:

1. Letendre, C.H., Nagaiah, K., and Guroff, G.: Brain amino acids. In Kumar, S. (ed.): Biochemistry of Brain, Oxford, Pergamon Press, 1979, pp. 339-378.

2. MacDonnell, P., Huff, K., Grouse, L., and Guroff, G.: Brain nucleic acids. In Kumar, S. (ed.): Biochemistry of Brain, Oxford, Pergamon Press, 1979, pp. 207-234.

3. Guroff, G.: Effects of inborn errors of metabolism on the nutrition of the brain. In Wurtman, R.J. and Wurtman, J.J. (eds.): Nutrition and the Brain, Vol. 4. New York, Raven Press, 1979, pp. 29-77.

4. Guroff, G.: Molecular Neurobiology, New York, Marcel Dekker, Inc., 1980, pp. 1-571.

5. Ikeno, T. and Guroff, G.: The effect of vasopressin on the activity of ornithine decarboxylase in rat brain and liver. J. Neurochem. 33: 973-975, 1979.

6. Huff, K.R. and Guroff, G.: Nerve growth factor-induced reduction in epidermal growth factor responsiveness and epidermal growth factor receptors in PC12 cells: An aspect of cell differentiation. Biochem. Biophys. Res. Commun., 89: 175-180, 1979.

7. Ikeno, T. and Guroff, G.: Growth regulation by nerve growth factor. Mol. Cell. Biochem. 28: 67-91, 1979.

8. Vinores, S. and Guroff, G.: Nerve growth factor: Mechanism of action. Ann. Rev. Biophys. Eng. 9: 223-257, 1980.

9. Guroff, G., Montgomery, P., Tolson, N., Lewis, M.E., and End, D.: The induction of ornithine decarboxylase by renin-free nerve growth factor. Proc. Natl. Acad. Sci. U.S.A., in press.

10. Yu, M.W., Tolson, N., and Guroff, G.: Increased phosphorylation of specific nuclear proteins in superior cervical ganglia and PC12 cells in response to nerve growth factor. J. Biol. Chem., in press.

11. Guroff, G.: Inborn errors of amino acid metabolism in relation to diet. Nutrition in Health and Disease, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00094-10 LDN

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Circadian, Stress, Light and Drug Influences (rat, hamster, rhesus monkey)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. C. Klein	Physiologist	LDN NICHD
OTHER:	J. L. Weller	Chemist	LDN NICHD
	S. Reppert	Cilinical Associate	NPMB NICHD

COOPERATING UNITS (if any)
R.Y. Moore, Dept. of Neurosciences, Univ. of CA, School of Medicine; R. Kvetnansky, LCS, NIMH; H. Hoffman, EBR, NICHD: M. Perlow and M. Mishkind, NIMH.

LAB/BRANCH
Laboroatory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to increase our understanding of the factors which regulate the metabolism of a neuroendocrine organ, the pineal gland. These investigations are conducted within a heirarchy of levels of biological organization, including the interactions of the pineal gland with environmental lighting, stress, drugs, endocrine organs, and the central nervous system. Studies related to the central nervous system also probe the fundamental nature of the biological clock governing biological rhythms.

Project Description:

Objectives: To determine how stress, central neural structures, environmental and pharmacological factors influence horomonal secretion by the pineal gland, and to study the fundamental nature of circadian rhythms.

Methods Employed: (1) To determine the central neural, environmental and pharmacological factors influencing the pineal gland, pineal glands are removed from treated animals and the activity of metabolites and enzymes are measured. In addition, the amount of melatonin in blood, CSF and the pineal gland is measured. The animals are treated with drugs, hormones, or experimental environmental regimes, including stress and constant darkness, and in some cases are surgically prepared by the precise destruction of small areas of the brain and neural structures thought to be part of the neuronal circuit regulating the pineal gland.

In addition, small areas of the brain known to be involved in the regulation of the pineal gland are removed and the amounts of neurotransmitters they contain are determined after experimental treatment.

(2) Circadian rhythms are studied by measuring the effects of destruction of brain areas on the circadian rhythm in pineal N-acetyltransferase activity, and in melatonin in the circulation.

Major Findings: (1) It has been found that the daily rhythm in CSF melatonin in the rhesus monkey is truly circadian in nature, persisting in constant darkness for six days. In addition, it has been found that light can cause a rapid decrease in melatonin in the CSF of the primate. These effects of light are apparent throughout the night. The opposite, however, is not true: exposure of animals to darkness during the day does not cause an increase in melatonin. (2) Analysis of the circadian rhythm in pineal melatonin in the Syrian hamster has revealed that this is truly circadian and in addition has the unique characteristic of being closely linked in time with the rhythm in running activity in this animal. (3) These studies have also revealed that the rhythm in melatonin in the hamster pituitary gland is strikingly different from the rhythm in melatonin production seen in other animals. In the hamster the period during which melatonin production takes place is short, being no more than four hours, and exhibits a sharp peak late in the night. In contrast in the monkey, the sheep and the rat the period during which melatonin is synthesized in the the hamster makes it possible for subtle differences in the timing of the occurance of melatonin production to be detected. This in part has led to a new concept of the action of melatonin on reproductive physiology that can be summarized as follows: "It's not how much, but when". The exciting new discovery has been made that the destruction of the suprachiasmatic nucleus in the primate has remarkable effects on the rhythm in CSF melatonin in the primate. Apparently in this sub-human primate as is ture of the rat, the suprachiasmatic nuclei play an important role in generating the daily rhythm in melatonin. (4) It has been found that the rhythm in melatonin in the rhesus

monkey can be shifted by shifting enviornmental lighting. Interestingly, we have found that there is entrainment within a one-day period when the rhythm is shifted 12 hours by extending the normal day period twelve hours, providing the animal with a continuous period 24 hours of constant light. In contrast when an animal is provided with an extended night period, providing 24 hours of constant darkness, the period required for the animal to entrain to the new lighting cycle is three days.

Significance to Biomedical Research and the Program of the Institute: A growing body of knowledge points to the pineal gland as the source of a compound that inhibits reproduction, and that this compound is melatonin. Large changes in melatonin production are regulated by N-acetyltransferase. Thus, an increase in understanding of how melatonin production is regulated provides a more complete description of the interaction of the pineal gland in reproductive physiology.

With knowledge in hand regarding the factors which regulate melatonin production, including drugs, environment, developmental and stress, it may be possible to regulate the production of this compound, and to predict when production would be enhanced.

Our studies on circadian rhythms in animals may have direct influence on the modern day problem of "jet lag". If we can alter the rate at which new circadian patterns are established in rats and hamsters after a shift in lighting schedules, it seems possible that this might be applied to humans to prevent "jet lag".

Proposed Course of Project: (1) Studies on the neural structures involved in the regulation of the pineal melatonin rhythm in the rhesus monkey will continue. (2) Experiments in the rhesus monkey will be performed of a pharmacological nature, designed to shed light on the transmitters involved in regulating pineal melatonin rhythm. These studies will include analysis of animals in which the superior cervical ganglia, which probably innervates the pineal gland, has been removed. This will allow the evaluation of the effects of administration of drugs on the pineal gland in this situation in which neural elements innervating the pineal gland are absent.

Publications:

1. Reppert, S., Perlow, M., and Klein, D.C.: CSF Melatonin. In J.H. Wood, ed.: Neurobiology of Cerebrospinal Fluid, Plenum Press, New York, (in press).

2. Perlow, M.J., Reppert, S.M., Tamarkin, L., Wyatt, R., and Klein, D.C.: Photic regulation of melatonin: Man and monkey are not the same. Brain Research 182, 211-216, 1980.

3. Reppert, S. and Kelin, D.C.: The pineal gland: basic and clinical aspects. In M. motta, Ed: Endocrine Functions of the Brain; Comprehensive Endocrinology IV, Raven Press, New York (in press).

4. Tamarkin, L., Reppert, S.M., Klein, D.C., Pratt, B., and Goldman, B.P.: Circadian nature of pineal melatonin in the Syrain hamster. Endocrinology 1980 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00095-10 LDN

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Transsynaptic Mechanisms in the Pineal Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	J.L. Weller	Biologist	LDN	NICHD
	L. Tamarkin	Guest Worker	LDN	NICHD
	D. Auerbach	Clinical Associate	NPMB	NICHD

COOPERATING UNITS (if any)
G. Aurbach, K. Kirk, C.L. Creveling, MDB, NIAMMD.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the transsynaptic mechanisms which are important in regulating metabolism of a neuroendocrine tissue, the pineal gland. The specific topics of interest include the interaction during differentiation of the pineal gland and innervating structures, the role of nerves in both releasing and taking up transmitters, the interaction of transmitters in transsynaptic regulation and the characterization of postsynaptic adrenergic receptors.

Project Description:

Objectives: To describe the transsynaptic mechanisms through which neuronal activity regulates biochemical activity in the pineal gland.

Methods Employed: (1) Cultures of pineal tissue are used to study the molecular mechanisms through which neuronal activity regulates biochemical activity. These preparations are treated with neurotransmitters, drugs, and hormones to determine their effects on pineal cell metabolism. (2) To study development, pineal glands are removed at various ages and the activity of metabolites and enzymes are measured. The animals are also treated with drugs known to block specific neural processes. (3) Beta-adrenergic receptors are characterized using a radioligand binding assay.

Major Findings: (1) We have found that the efflux of taurine from the pineal gland is regulated by adrenergic-cyclic AMP mechanism. The treatment of glands with norepinephrine results in a 100-fold increase in the rate of efflux of taurine. This release appears to be related to changes in membrane potential and the driving force behind the release is not the taurine concentration gradient. (2) The beta-adrenergic receptors on pineal cells have been studied developmentally and appear to develop during the perinatal period. The relationship of the appearance of receptors, of adenylate cyclase, and of adrenergic cyclic AMP stimulated N-acetyltransferase have been determined. (3) Ring-fluronated derivatives of norepinephrine were found to be equipotent or less potent that norepinephrine as regards their ability to elevate cyclic AMP. This effect was found to be a reflection of the binding of the compound to the beta-adrenergic receptor.

Significance to Biomedical Research and the Program of the Institute: The pineal gland represents one of the most attractive models of adrenergic control mechanisms presently available. Our understanding of the transsynaptic mechanisms involved in regulating this tissue will lead to a fuller understanding of neural functions in humans and especially in the development of neural control mechanisms. It may be possible, with an increased understanding of the specific structures and proteins involved in neural transmission and neural biochemical transduction, to identify a set of specific proteins required for normal adrenergic functioning. With these markers in hand it may be possible to evaluate neural disease against these standards and thus to fully diagnose these neural diseases on a molecular basis. This should provide a thorough understanding of the nature of alterations in neural development and mental retardation and also help provide a means of designing strategies to solve problems associated with these issues.

Proposed Course of Project: (1) The effect of taurine on transsynaptic control of adrenergic function will be studied. (2) The role of cyclic GMP in pineal function will be studied. (3) The regulation of taurine efflux will be investigated further. (4) The development of the adrenergic control of taurine efflux will be studied.

Publications:

1. Wheler, G.H.T. and Klein, D.C.: Function and regulation of taurine in the pineal gland. in: S.I. Baskin, J.J. Kocsis, and S.W. Shaffer, eds.: Actions of Taurine on Excitable Tissues, Spectrum Press, New York (in press).

2. Wheler, G.H.T. and Klein, D.C.: Taurine release from the pineal gland is stimulated by a beta-adrenergic mechanism. Brain Research 187, 155-164, 1980.

3. Wheler, G.H.T. and Klein, D.C.: Cyclic AMP induced release of [^{14}C]-taurine from pinealocytes. Biochemical Biophysical Research Communications 90, 22-27, 1980.

4. Klein, D.C., Auerbach, D., Namboodiri, M.A.A., Wheler, G.H.T.: Indole metabolism in the mammalian pineal gland. In Pineal Gland: Anatomy and Biochemistry, Ed. R. Reiter, CRC Press, 1980 (in press).

5. Auerbach, D., Klein, D.C., Kirk, D., Creveling, C.R.: The effect of fluorine derivatives of norepinephrine on the adrenergic stimulation of cyclic AMP in intact pinealocytes. Biochemical Pharmacology, 1980 (in press).

6. Auerbach, D.A., Weller, J., and Klein, D.C.: Perinatal development of the pineal β-adrenergic adenylate cyclase control system. Journal of Neurochemistry, 1980 (in press).

7. Auerbach, D.A., Woodard, Auerbach, G.D., and Klein, D.C.: Neonatal rat pinealocytes characteristis of typical and atypical ^{125}I-iodohydroxybenzylpindolol binding and cyclic AMP accumulation. Endocrinology, 1980 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00096-10 LDN

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Intracellular Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	A. Namboodiri	Visiting Associate	LDN	NICHD
	J.L. Weller	Biologist	LDN	NICHD

COOPERATING UNITS (if any)
K.L. Kirk, LC, NIAMMD; J. Pierce, OD, NHLI; G. Kapatos, LNC, NIMH.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations have centered on understanding on a molecular level the intracellular regulation of metabolism in a neuroendocrine organ, the pineal gland. The topics of specific interest include the factors regulating cyclic AMP production, the function of cyclic AMP, the control of synthesis of pineal N-acetyltransferase activity, the control of activation and inactivation of this enzyme and related enzymes, and the role of mRNA synthesis in the regulation of the activity of N-acetyltransferase activity.

Project Description:

Objectives: To describe the intracellular mechanisms through which neuronal activity regulates biochemical activity in the pineal gland.

Methods Employed: (1) The experimental systems used to study the intracellular mechanisms through which neuronal activity regulates biochemical activity are cultures of pineal glands and broken cell preparations. The intact preparations are treated with neurotransmitters, drugs, and hormones to determine their effects on pineal cell metabolism. Other measures of cell function are studied, including the generation of cyclic nucleotides, the production of new protein, the production of new RNA, the activity of specific enzymes, and the production of metabolic products, including several tryptophan derivatives. (2) Broken cell preparations are used to study the regulation of pineal N-acetyltransferase activity. In addition this enzyme is purified.

Major Findings: (1) Substantial progress has been made in purifying N-acetyltransferase from the sheep. The major advances have been the incorporation of techniques based on disulfide exchange. This coupled with affinity and hydrophobic chromatography yields a purified preparation of the enzyme. (2) The activity of pineal acetyl CoA hydrolase, which previously have been found to be activated by disulfide exchange, has been found to be activiated in cells by a similar mechanism. (3) There is clear evidence that the activity of pineal N-acetyltransferase, and the activity of acetyl CoA hydrolase can be regulated by disulfide exchange. The participating disulfide compounds include small molecules such as cystamine. A new exciting discovery was that disulfide exchange reactions involving these enzymes and a number of biologically occurring peptides also results in distinct and rapid changes in activity in both enzymes. In addition, the specificity and potency of each peptide is different suggesting that peptides might be physiologically involved in the regulation of these enzymes through disulfide exchange mechanisms. (4) It has been possible to isolate two active bands of pineal N-acetyltransferase using electrophoretic methods (PAGE). These bands are of distinct molecular size, as indicated by gel exclusion chromatography, and both bands contain similar amounts of N-acetyltransferase activity. These findings indicate that in the sheep pineal gland there are two active forms of N-acetyltransferase that may be present within the cell.

Significance to Biomedical Research and the Program of the Institute: The pineal gland offers an attractive model system for the study of the neural control of gene expression. Although other neural systems are available, none is capable of exhibiting such large and rapid changes in the expression of single gene as is the pineal gland. In addition, with the knowledge and confidence that the increase in pineal N-acetyltransferase depends upon the synthesis of new messenger RNA, it is now appropriate to determine whether or not the neural regulation of this gene, which must be considered to be a high information-low abundance gene, is similar to gene regulation seen in other tissue, as studied through the analysis of major gene products. Our understanding of the factors which control rapid changes in enzyme activity

as a function of neural stimulation will be of great importance in understanding the neural control of metabolism in general. It is highly possible that transmitters control many enzymes in a manner similar to that in which norepinephrine controls N-acetyltransferase. Knowing what we know now about the regulation of pineal N-acetyltransferase, we may be able to use these lessons to study and analyze the regulation of enzyme activity in other neurally-controlled tissues, including neurons from the central nervous system.

Proposed Course of Project: (1) The isolation and characterization of pineal N-acetyltranasferase will continue to be pursued. (2) Efforts to generate antibodies against pineal N-acetyltransferase will be continued. (3) The role of disulfide exchange and the regulation of pineal N-acetyltransferase and acetyl CoA hydrolase will be investigated. (4) The molecular mechanism involved in the regulation of both enzymes will be investigated. (5) The possible role of peptides in the regulation of this enzyme will be investigated. (6) The regulation of pineal biopterin will be investigated.

Publications:

1. Namboodiri, M.A.A., Nakai, C., and Klein, D.C.: Effect of selected treatments on the stability and activity of pineal serotonin N-acetyltransferase. Journal of Neurochemistry 33, 807-810, 1979.

2. Namboodiri, M.A.A., and Klein, D.C.: Activation of pineal acetyl-CoA hydrolase activity by cystamine. Biochemical and Biophysical Research Communications 91, 1166-1173, 1979.

3. Namboodiri, M.A.A., Klein, D.C.: Evidence of inactivation of pineal N-acetyltransferase by protein thiol-disulfide exchange. Journal of Biological Chemistry, 1980 (in press).

4. Namboodiri, M.A.A., Weller, J.L., and Klein, D.C.: Rapid and reversible activation of pineal acetyl CoA hydrolase in intact pineal cells by disulfide exchange. Biochemical and Biophysical Research Communications, (in press).

5. Klein, D.C. and Namboodiri, M.A.A.: On the pineal setotonin N-acetyltransferase trail: Cystamine now lights the way. Nature, 1980 (in press).

6. Klein, D.C., Weller, J.L., Auerbach, D., and Namboodiri, M.A.A.; Regulation of pineal N-acetyltransferase: Focus on "turn-off". In Enzymes and Transmitters in Mental Disease (ed. M. Youdim and E. Usdin), 1980 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00097-10 LDN

PERIOD COVERED
October 1, 1979 through September 31, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of Neuroendocrine Metabolism: Melatonin Physiology (rat, hamster, rhesus monkey)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	S. Reppert	Clinical Associate	NPMB	NICHD
	L. Tamarkin	Guest Worker	LDN	NICHD

COOPERATING UNITS (if any)
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Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Efforts are directed at fully describing the physiology of a putative neuroendocrine hormone, melatonin. Current interest is centered on describing the amount of this compound present in body fluids throughout life.

Project Description:

Objectives: To study the factors regulating the amount of circulating melatonin, a putative pineal hormone during life.

Methods Employed: (1) Melatonin metabolism, fate, and distribution is studied using [^{3}H]melatonin. Thin layer chromatography is used to isolate [^{3}H]melatonin and its metabolites. (2) Melatonin is measured by radioimmunoassay.

Major Findings: (1) In studies involving pregnant rhesus monkeys and pregnant ewe it was discovered that radioactive melatonin is rapidly transfered across the placenta. In addition, using low concentrations of melatonin it was also found that the dynamics of transfer were such a nature as to make it highly probable that the rhythm in melatonin in the mother would be transmitted to the fetus quite rapidly. In associated studies, it was also found that the fetal pineal gland of the monkey and sheep has the ability to make melatonin. Thus, it would seem highly probable that thoughout the development of the fetus that melatonin from either the mother or from the fetus itself would be available. (2) The development of the rhythm in pineal melatonin has been studied in several species including the hamster and the rat. In both animals the rhythm in melatonin is absent at birth and developes at about the end of the second week of life. The development of this rhythm closely parallels the development of enzymatic machinery required for the last step in melatonin production, the enzyme involved being hydroxyindole-O-methyltransferase. In addition, it has been found that the photoic regulation of the melatonin rhythm in the rat is apparent as soon as the animal is capable of synthesizing melatonin on a rhythmic basis. This would indicate that the neural connections between the eye and the pineal gland are complete by the end of the second week of life.

Significance to Biomedical Research and the Program of the Institute: A growing body of knowledge points to the pineal gland as the source of a compound that inhibits reproduction, and that this compound is melatonin. An increase in our understanding of how melatonin production is regulated, when production is first regulated, when production is initiated, the relative importance of melatonin from mother's milk, the fate of melatonin, and the biological effects of melatonin analogs and metabolites is in accord with the Institute's mission to study reproduction.

Proposed Course of the Project: To determine if there are genetically distinct varients available among the genetic strains of mice in which melatonin synthesis is altered.

Publications:

1. Reppert, S., Chez, R.A., Anderson, A., and Klein, D.C.: Maternal-fetal transfer of melatonin in the non-human primate. Pediatric Research 13, 788-790, 1979.

2. Klein, D.C., Namboodiri, M.A.A., and Auerbach, D.A.: The melatonin rhythm generating system: Developmental aspects. Life Sciences 1980 (in press).

3. Tamarkin, L., Reppert, S.M., Orloff, D.J., Klein, D.C., Yellon, S.M., and Goldman, B.D.: Ontogeny of the pineal melatonin rhythm in the Syrian (Mesocricetus auratus) and Siberian (Phodopus sungorus) hamsters and in the rat. Endocrinology, 1980 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00700-03 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Cell interactions in synaptogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.N. Christian	Staff Fellow	LDN NICHD
	M.P. Daniels	Biologist	LBG NHLBI
OTHER:	P.G. Nelson	Chief, LDN, NICHD	LDN NICHD
	H.C. Bauer	Guest Worker	LDN NICHD
	J. Prives	Dept. Anat. Sci.	SUNY
	P. Pudimat	Bio. Lab. Tech.	LDN NICHD
	M. Jackson	Guest Worker	LB NINCDS
	D. Axelrod	Bioph. Res. Div.	Univ. of Michigan
	S. Hasegawa	Guest Worker	LDN, NICHD

COOPERATING UNITS (if any)
Laboratory of Biochemical Genetics, NHLBI; Laboratory of Biophysics, NINCDS: Biophysics Research Division, University of Michigan; Dept. of Anatomical Sciences, SUNY, Stony Brook

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is the investigation of the cellular and molecular interactions during synaptogenesis, and is a continuation of the project described in the previous annual report. We have taken the interaction between neurons and muscle cells grown in vitro as a model system of the development of the neuromuscular junction.

We have found that a factor or factors produced by neuronal cells, when added to muscle cells: 1) aggregates acetylcholine receptors (AChR), 2) causes the lateral immobility of AChR, 3) promotes the attachment of AChR to the myotube cytoskeleton, 4) decreases the rate of degradation of AChR.

PHS-6040
(Rev. 10-76)

Objectives: To characterize the early molecular events in the cell cell recognition involved in synapse formation. We will focus on those events which occur in the postsynaptic muscle cell when innervated by nerve. We will relate the neuronal modulation of the AChR topography, metabolism, and physiological kinetics to a common sequential cellular mechanism. We will isolate the neuronal factor or factors responsible for the modulation of the post-synaptic receptors, and characterize the manner in which they interract with the muscle cell. We wish to test the molecular specificity of these effects for various cell and receptor types, as an approach to testing and explaining the synaptic specificity which arises in embryogenesis.

Methods: Both pre- and post-synaptic cells are grown and interracted in vitro, with standard tissue culture procedures. In order to obtain large quantities of the AChR aggregation factor, a continuous neuronal cell line has been cultured in bulk, or the factor has been extracted from embryonic brain tissue.

In order to study the spatial distribution, metabolism, or detergent extraction kinetics of muscle cell AChR, alpha-bungarotoxin, a specific probe for the AChR has been tagged with various probes, including I^{125}, rhodamine, fluorescein, horse radish peroxidase, and ferritin.

The ultrastructural correlates of AChR aggregation and nerve muscle interraction have been studied by preparing cultured muscle cells for scanning or transmission electron microscopy. Methods have been developed to study the topographic behavior of the AChR in living cells and to relocate the same area of membrane by electron microscopy.

A standard method of the detergent extraction of AChR from muscle cells has been developed, and used for the characterization of the AChR attachment to muscle cell cytoskeleton, and for the further purification of the neuronal factor which promotes this attachment.

Standard electrophysiological techniques, including noise analysis and and the direct recording of single AChR channels have been used to studying the pharmacological properties of the AChR and to characterize the effect of neuronal factors on AChR channel kinetics.

Major Findings: Medium conditioned by NG108-15 cells causes a significant increase in the number of AChR aggregates per myotube. This effect, observed within 4 hours, is temperature dependent, requires Calcium, and is blocked by colchicine, but is not blocked by cytochalasin-B. To study the role of the cytoskeleton in AChR aggregation, the rate of detergent extraction of the AChR was assessed in similar pharmacological experiments. The decrease in the rate of detergent extraction produced by treatment of myotubes with NG108-15 cell conditioned medium was also temperature, and Calcium dependent, but was blocked by cytochalasin-B and not by colchicine. The results suggest a two step process in the neuronal modulation of muscle cell AChR, with

the topographic redistribution of AChR dependent on the integrity of myotube microtubules, and the AChR anchorage dependent on microfilaments. Two myotube systems modulated by NG108-15 cell conditioned medium are also suggested by effects of various agents which cross-link integral membrane proteins. The non-specific glycoprotein cross-linker Concanavalin-A and antibodies directed against the AChR both decrease the rate of AChR detergent extraction. However, cross-linking by antibodies has little effect on AChR aggregation, but aggregation is blocked by Concanavalin-A.

Electron microscopy was used to determine the identity and arrangement of cytoskeletal elements to which the AChR may be attached. Following the detergent extraction of myotubes in which there is a retention of a large proportion of AChR with the myotube cytoskeleton, there is nearly a complete extraction of the plasma membrane, mitochondrial, endoplasmic reticulum and nuclear membranes, as well as the "ground cytoplasm". The remaining structures included the nuclei, polyribosomes, myofibrils, and arrays of cytoskeletal elements, including microtubules, intermediate filaments and microfilaments. A fibrous plasma lamina, located directly beneath the extracted plasma membrane, was composed primarily of microfilaments. Developmental studies on both cultured chick and rat muscle cells demonstrated an increase in the complexity and extent of the plasma lamina, which correlates with a development increase in the resistance of the AChR to detergent extraction.

Metabolic stability is a prominent characteristic of the AChR found at the mature neuromuscular junction. The metabolic stability of the AChR of cultured myotubes was found to be modulated by medium conditioned by neuronal cells. In untreated myotube cultures the degradation of AChR follows first order kinetics, with a half-time of 22.5 hours. In myotube cultures treated with NG108-15 cell conditioned medium, the half-time of degradation was increased by one third, with some increase in the rate of insertion of newly synthesized AChR into the myotube membrane. The effect of both neuronal modulations is to increase the concentration of AChR in the myotube membrane. Adenosine and adenosine 5'-triphosphate (ATP) were found to decrease the rate of myotube AChR degradation. were found to decrease the rate of myotube AChR degradation. The kinetics of single AChR channels was characterized. In cultured rat myotubes, there are two populations of AChR with respect to mean channel open time. The cholinergic antogonist d-tubocurarine preferentially blocks the cholinergic activation of the long mean channel open time channels. This suggests a correlation between the properties of the receptor acetylcholine binding site and the kinetics of its associated channel.

Proposed Course: To purify and characterize the factor(s) which produce the various described effects on the AChR of muscle cells, and to relate these state transitions to a common sequential cellular mechanism. To extend our analysis of the neuronal regulation of receptor properties to other receptors on the muscle cell and to receptors on other types of cells.

Publications:

1. Bauer, H.C., Daniels, M.P., Pudimat, P.A., Jacques, L., Sugiyama, H. and Christian, C.N.: Characterization and partial purification of a neuronal factor which increases acetylcholine receptor aggregation on cultured muscle cells. Brain Res., in press.

2. Christian, C.N., Bergey, G.K., Daniels, M.D., and Nelson, P.G.: Synapse formation and stabilization in neuronal development. J. Exp. Biology, in press.

3. Prives, J., Christian, C., Penman, S. and Olden, K.: Neuronal regulation of muscle acetylcholine receptors: role of muscle cytoskeleton and receptor carbohydrate. In: Giacobini, et al. (ed.): Tissue Culture in Neurobiology, New York, Raven Press, pp.35-52, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00701-01 LDN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Anticonvulsants on Cyclic Nucleotides in Cultured Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. Study Staff Fellow LDN NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Betheada, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cultured mouse neuroblastoma cells were used to determine the effects of anticonvulsants, particularly phenytoin, on cyclic GMP and membrane electrical events. Neuroblastoma cells respond to depolarization (as well as to activation of certain membrane receptors) by increasing cyclic GMP synthesis in a manner apparently similar to that seen in brain slices. We have found that phenytoin and other anticonvulsants active against major seizure types inhibit cGMP increases in neuroblastoma cells by a mechanism involving blockade of calcium influx or translocation. This property is not shared by drugs which are active only against absence of petit mal seizures. These findings may help provide a basis for understanding the biochemical changes associated with convulsive activity and the mechanism of action of anticonvulsant drugs.

Project Description:

Objectives: Analysis of the action of phenytoin and other anticonvulsants on cyclic nucleotide metabolism and membrane electrophysiology in cultured neuroblastoma cells.

Methods Employed: Monolayer cultures of mouse neuroblastoma cells (clone N1E-115) are used as a simplified neuronal model system which allows both electrophysiological analysis of individual cells and biochemical analysis of the culture as a whole, due to its relative homogeneity. Levels of cyclic GMP in the cultures are measured by radioimmunoassay and levels of cyclic AMP by a protein kinase binding assay, under conditions where the levels are an indication of synthesis rates. Membrane electrical events are measured by intracellular recording using conventional techniques of potential measurement.

Major Findings: It was found that phenytoin in concentrations which are close to optimal clinical blood levels inhibits increases in cyclic GMP which are caused by either membrane depolarization (by high potassium medium) or by stimulation of muscarinic or histamine H_1 receptors. The action of phenytoin appears to be at the level of the cell membrane rather than on the enzyme system responsible for synthesis and degradation of cyclic GMP.

Furthermore, it was determined that phenytoin probably inhibits cyclic GMP responses by inhibiting calcium influx (and perhaps intracellular translocation of calcium). Calcium is required for both depolarization-induced and receptor activation-induced cyclic GMP responses and the effect of phenytoin can be overcome by causing calcium influx artifically using the ionophore A23187. Phenytoin does not alter the apparent affinity of acetylcholine for the muscarinic receptor. However, the blockade of the muscarinic response can be overcome by increasing calcium in the medium. The relationship between calcium and phenytoin is non-competitive for the depolarization-induced cyclic GMP response.

Other anticonvulsants effective against major tonic-clonic and cortical focal seizures, phenobarbital and diazepam, inhibit the cGMP responses in these cells. Drugs effective only on absence seizures, trimethadione and ethosuximide, had no effect, suggesting that the blockade of calcium channels or the subsequent inhibition of cyclic GMP increases may be related to this type of anticonvulsant activity.

Electrophysiological studies have supported the calcium channel blockade hypothesis. Phenytoin had no effect on resting membrane potential or input resistance in concentrations which had a significant effect on cyclic GMP. Calcium-dependent portions of the action potential were inhibited, but to an extent which appeared to be slightly less than expected from the cyclic GMP results, although only a few cells were examined and a reliable dose response curve was not produced. Tetrodotoxin-sensitive, sodium-dependent components of the action potential were also inhibited by

phenytoin but to a lesser extent and at higher concentrations than for the calcium components.

Significance to Biomedical Research and the Program of the Institute: The study of neuronal properties and their influences by drugs using cell lines of neuronal origin provides a means for direct control of cell environment, observation of growth and morphology, and both electrophysiological and biochemical analysis on a homogeneous system. In this case, the inhibition of cGMP increases by phenytoin was able to be analyzed and a connection made with membrane electrical events. Since cyclic nucleotides can both influence electrophysiological events and be influenced by them, they may represent important regulatory agents in excitability. The establishment of a mechanism by which anticonvulsants can interact with this system may help provide a better scientific basis for the understanding of anticonvulsant actions.

In addition, these results may also provide a clue to understand the effects of anticonvulsants on cell viability, since both cyclic nucleotides and calcium may be involved in cell growth control.

Publications

None

NICHD ANNUAL REPORT
October 1, 1979 through September 30, 1980

Laboratory of Molecular Genetics

The Laboratory of Molecular Genetics conducts research directed towards understanding the molecular processes involved in the transmission of genetic information and its regulated expression during growth and differentiation. A variety of model systems are under investigation, ranging from bacterial and animal viruses to complex vertebrate regulatory responses involving the synthesis of specific antibodies and the development of specific organ systems. Taken together, the Laboratory joins active workers in the areas of genetics, biochemistry, electron microscopy, immunology, virology and cellular biology to bring their skills to bear on problems of mutual interest. At present the Laboratory is comprised of ten research groups disbributed among six sections.

I. Section on Gene Organization and Control

A. Studies have been directed towards understanding the regulated expression and organization of two sets of mammalian genes, those involved with globin and those involved with immunoglobulin production. In earlier reporting periods we developed strategies for cloning a number of genes derived from each system. During the past year we have progressed considerably with both a structural and functional analysis of these genes. By comparing the detailed structures of alpha and beta globin genes we have been able to identify regions within them that are concerned with critical regulatory functions such as transcription promotion, polyA addition and RNA splicing. We have further introduced segments of these genes into hybrid SV40 virus and into cell-free transcriptional systems to locate specific promoter regions and (in the case of SV40 hybrids) to demonstrate the requirement that a gene be interrupted in order to be expressed. We have also identified a group of pseudogenes, including one that entirely lacks intervening sequences and have speculated on its origin. In the immunoglobulin system, we have shown how immunoglobulin gene diversity arises through a site-specific recombination event that joins one of many V-region genes to one of four J region segments. We have also shown that this reaction can occur at several different crossover points so as to generate additional diversity in a critical region of the light and heavy chains. We also have characterized the V/J recombination event as one that is accompanied by deletion of both V- and J-region segments. (Dr. Philip Leder -- Principal Investigator)

B. Studies have been directed towards understanding the regulatory pathways controlling the antibody response at the cellular level. The integrated roles of regulatory T-lymphocytes, macrophages and responder B-lymphocytes are being delineated in vitro using cells obtained from athymic (nu/nu) mice and normal (+/nu or +/+) NFR/N mice. Microbial and plant agents are known to variously affect the immune system in animals and humans. This natural circumstance is being exploited in mice in order to understand pathways toward the regulation of the immune system. A streptococcal protein (SPE, an exotoxin) was shown to profoundly cancel the activity of normal suppressor T-lymphocytes in experimental antibody responses to haptenic immunogens. In recent experiments we have been able to distinguish and separate two populations of T-lymphocytes. One responds to SPE while the other does not. This has allowed us to formulate two alternative models of the development of immune regulation by T-cells. One is that suppression and help are different functions associated with the same T-cells but at different stages of their development. The other is that suppression and help are different functions of different T-cell lineages. In the former, SPE could drive the development selectively along the helper course. In the latter, SPE could merely inactivate the suppressor population causing an unbalanced helper activity. In order to distinguish between these alternate pathways of T-cell development and immune regulation, T-cell hybridomas have been developed, and cloned in order to ascertain the regulatory pathways of T-cells. A helper T-cell hybridoma has been constructed and cloned. Other T-cell hybridomas have been identified which possess graded activity profiles from suppression to elevated help. Thus this approach may stabilize the T-cell phenotypes so as to ascertain the T-cell circuitry involved in regulation of the immune sytem. (Dr. Edgar E. Hanna -- Principal Investigator)

C. Studies have been directed towards understanding the molecular events involved in the replication and assembly of RNA tumor viruses. Stability of viral messages and utilization of intracellular viral RNA species for encapsidation or messenger function are being investigated under conditions which block ongoing synthesis of viral RNA (actinomycin D system). Liquid hybridization experiments with a murine leukemia virus cDNA probe demonstrate that after 8-12 hours of actinomycin D treatment, virus-specific RNA sequences in virions are reduced by 90%, whereas cellular sequences are decreased by only 2-fold. These data suggest that viral messages have a longer half-life than viral RNA which is ultimately packaged into virions. Efforts are now being directed towards identifying the specific viral RNA species which react with the cDNA probe. Work has continued on mechanisms responsible for selective encapsidation of host tRNAs and the role of reverse transcriptase in selecting the tRNA primer for RNA-directed DNA synthesis. Analysis of a newly obtained nonconditional murine leukemia virus mutant which lacks reverse transcriptase has confirmed the finding that in the absence of the enzyme, the free 4S RNA of virions is deficient in tRNA primer (proline tRNA), but not in other viral tRNA species. Evidence that the mutant has normal levels of tRNA primer associated with 35S genomic RNA suggests that reverse transcriptase does not participate in the site-specific binding of the tRNA primer to the genome. Finally, a system has been developed in which chromosomes are used to transfer viral genetic information to uninfected cells.
(Dr. Judith G. Levin -- Principal Investigator)

D. Studies have been directed towards understanding the organization of genes encoding the transplantation antigens (mouse major histocompatibility antigens). We expect that recombinant DNA techniques will eventually allow a much better understanding of this multi-gene complex. The initial step in this approach requires the molecular cloning of the mRNA sequences produced from these genes. A modification of existing technologies for cloning mRNA sequences has been required because the mRNAs for histocompatability antigens comprise a small fraction of the total cellular mRNA. We are currently involved in cloning and characterizing the mRNAs for beta-2 microglobulin and the mouse histocompatability antigens, H-2D and H-2K. (Dr. Jonathan Seidman - Principal Investigator)

II. Section on Cellular Differentiation

Studies have been directed towards understanding the molecular events related to crystallin gene expression and protein synthesis in the vertebrate lens during development and disease (cataract). Emphasis has been given to identifying and characterizing the δ-crystallin genes. Chicken gene sequences from two δ-crystallin genes with 14 to 15 intervening sequences have been cloned and compared by restriction mapping and electron microscopy, opening the way for detailed studies on δ-crystallin gene expression during lens development. Experiments with ts Rous sarcoma virus have dissociated changes in lens epithelial cell growth and morphology from δ-crystallin gene expression, which is strongly reduced if not eliminated in the transformed cells. Studies on β-crystallin gene expression in the chicken lens have also been initiated. β-Crystallin has been shown to consist of a family of related polypeptides which are differentially synthesized during lens cell differentiation. Clones of α-, β- and γ-crystallin cDNAs have been obtained from mice and will be utilized to study crystallin genes and quantify crystallin mRNAs in this organism. Finally, investigations on hereditary cataracts in Nakano and Philly mice have supported the idea, developed earlier in this Section, that changes in the concentrations of intralenticular electrolytes reduce crystallin synthesis in osmotic cataracts. In vitro translation studies revealed that the initial reduction of crystallin synthesis in these cataracts is associated with poor utilization of persistent crystallin mRNAs, and that crystallin synthesis may be stimulated by increasing the K+ and decreasing the Na+ concentrations within the cultured lenses. (Dr. Joram Piatigorsky - Principal Investigator)

III. Section on Molecular Structure

Studies have been directed towards understanding the structure of animal virus genomes and the way viruses gain control of the host cell's molecular processes. Research has been concentrated in three major areas: poliovirus replication, early events in adenovirus replication, and methodology. Poliovirus genomes have been mapped electron microscopically, biochemically and genetically. A map of the positions of 76 RNase T1-resistant oligonucleotides has been correlated with the positions of secondary structure features

detected by electron microscopy and ribonuclease III digestion. Translational initiation-specific ribosome binding sites have been positioned at three major sites at both ends and internally in the genome. Cleavage, binding and translation in vitro has been compared with the poliovirus polyprotein map and shows a remarkable correlation of these sites with known boundaries on the mature virus proteins derived from a large polyprotein. Adenovirus interactions with the host cell have been analyzed by careful subcellular localization of the early proteins and analysis of their biosynthesis, and post-translational modification. Most of the adeno virus early proteins are intimately associated with the cytoskeleton of the cell. Pronounced morphological changes in the nucleus of the cell occur as the early proteins are expressed. Nucleic acid analysis programs have been devised to search for promoter, splice and attachment sites. Calculations are being programmed to allow interaction of the graphic matrix approach with combinatoric approaches to predict secondary structures for sequences of size typical of mRNAs.
(Dr. Jacob V. Maizel, Jr. -- Principal Investigator)

IV. Section on Animal Viruses

Studies have been directed towards understanding early adenovirus gene expression and control. Fine mapping of nuclear and cytoplasmic viral RNAs in the electron microscope has revealed a complex pattern of transcription and processing generating sets of mRNAs corresponding to at least six separate early regions of the adenovirus genome. We have established that functions coded by these early adenovirus mRNAs are necessary and sufficient to promote growth of the defective adeno-associated viruses, members of the parvovirus group. In addition, we have established and mapped the structures of parvovirus mRNAs. The structure of SV40 sequences integrated in early region 3 of adenovirus type 2 has been the topic of a collaborative effort. This virus exhibits a characteristic, host-dependent modification of its genomic DNA sequences and thus represents a useful model for studies of the molecular events that accompany host-virus adaptation.
(Dr. Heiner Westphal - Principal Investigator)

V. Section on Molecular Regulation

A. Studies have been directed towards understanding how cells coordinate the expression of their genome during growth and in particular how the regulatory nucleotide ppGpp participates in this control. Evidence indicates that ppGpp probably controls the initiation of transcription of rRNA, most tRNA and about half of the remaining structural genes in E. coli. There are two genes mediating ppGpp synthesis called relA and relS. We have discovered relS mutants in a relA background and thereby derived the first bacterial

strain totally incapable of ppGpp synthesis. This double mutant dies during amino acid starvation but grows normally under nutritionally adequate conditions. Interestingly, the combination of relS mutation with a wild type relA+ gene was found to be lethal. All together these studies have proven that ppGpp is a dispensable metabolite except for regulatory functions. Measurements of how ppGpp exerts its regulatory influence have been approached by development of a new system that uses a promoter fused to a detector gene (galactokinase) such that the fused DNA fragment can be moved easily between a plasmid vector and a lambda phage derivative. This allows structure activity investigations of the regulation of the ribosomal promoter region. We have also devised a method of enhancing the sensitivity of in vitro assays of gene expression of linear DNA fragments at least 100-fold. This allows measurements with small fragments that were previously either impossible or cumbersome because of the large amount of fragment required for assay.
(Dr. C. Michael Cashel -- Principal Investigator)

B. Studies have been directed toward understanding the role of certain ribonucleases in processing RNA. Studies of the processing of ribosomal RNA from chick embryos by RNase DII, an enzyme first isolated in this laboratory from chick embryos, has centered on the primary and secondary structure of the sequences involved in processing. Restriction enzyme analysis and sequence determination suggest that at least one of the cleavage sites is formed by the 3' terminus of 5.8S rRNA forming a stable duplex with the 5' terminus of 28S rRNA and cleavage occurs at--or near--the mature ends. 5.8S rRNA can exist in the precursor form in two alternate forms. One form occurs if the 5.8S rRNA hydrogen bonds with itself while a second interaction occurs when 5.8S rRNA interacts with the region of the precursor containing the sequences for 28S rRNA. Either form could easily be a site for recognition of RNase DII.
(Dr. Robert J. Crouch - Principal Investigator)

VI. Section on Microbial Genetics

Studies have been directed towards understanding the mechanisms by which a virus, the bacteriophage λ, inserts its chromosome into and excises it from the chromosome of its host. Both insertion and excision are examples of a general process called site-specific recombination that involves interactions between specific recombination proteins and "attachment" sites on the DNA substrate. The attachment sites are complex and diverse: that of the phage is large and contains specialized subregions, while that of the host is smaller and simpler. Several mutations in both of these sites have been isolated, characterized, and sequenced. With this data it has been possible to test and to reject two simple models of attachment site function. As more mutations are studied, it should be possible to determine with precision the importance of specific nucleotides in the protein-attachment site interaction

and the biochemical function of attachment site subregions. A phage mutation that appears to increase the production of a central recombination protein, integrase, is also being studied; it promises to shed light on the roles of the other recombination proteins and on the basis of site specificity. (Dr. Robert A. Weisberg - Principal Investigator)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00066-11 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Control Mechanims in Temperate Bacteriophage λ

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Weisberg	Microbiologist	LMG NICHD
	Christine Gritzmacher	Staff Fellow	LMG NICHD
	Dhruba Chattoraj	Visiting Fellow	LMG NICHD
	John Hays	IPA	LMG NICHD

COOPERATING UNITS (if any) Dr. Howard Nash, Lab. of Neurochemistry, NIMH; L. Enquist, Lab. of Molecular Virology, NCI; A. Landy, Brown University, Providence, R.I.; T. Platt, Yale University, New Haven, Conn.

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Microbial Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long term goal of this project is to determine the mechanism of recombination of virus DNA with the host chromosome. Recombination between virus and host involves specialized regions in each DNA called attachment sites. The locus of crossing-over lies within the attachment sites, specifically in a 15 bp region of homology called the core. Where within the core does polynucleotide strand breakage and rejoining actually occur? In collaboration with A. Landy, we have found that such breakage and rejoining can occur at two core positions (between bases -2 and 0, and between bases 4 and 5, where position 0 is the core center). We are further analyzing the function of the virus attachment site by isolation and sequencing of point mutations. Our initial results suggest that many of the bases within the attachment site can be changed without affecting site function. We are continuing our analysis of a virus mutant that increases the activity of integrase, the enzyme that promotes virus-host recomeination. Our results suggest that the mutation is located upstream of the DNA region that encodes the protein and may therefore act to increase the amount of the enzyme.

Project Description:

Objective: To understand the mechanism of virus-host recombination as exemplified by the insertion of the bacteriophage λ genome into the Escherichia coli chromosome.

Major Findings:

1. In collaboration with A. Landy and L. Enquist we have determined the basis of imprecise excision of the λ chromosome from a secondary attachment in the bacterial chromosome. (Imprecise excision differs from precise excision in that the original sequence of nucleotides is not restored after a cycle of insertion and excision.) The nucleotide sequence around the crossover points during a cycle of insertion and excision are shown below.

-3	-2	-1	0	+1	+2	+3	+4	+5	
T	T	T	T	A	T	C	C	T	- virus
G	*T*	*T*	*T*	*T*	*C*	*A*	*A*	*A*	- host
				↓					
T	T	T	T	A	T	A	C	A	Inserted
G	*T*	*T*	*T*	*T*	*C*	*A*	*A*	T	virus
				↓					
T	T	T	T	*T*	*C*	*A*	*A*	T	- Excised virus
G	*T*	*T*	*T*	A	T	A	C	*A*	- Excised hot

Insertion occurs by crossing-over between positions +4 and +5, and excision occurs by crossing-over between positions -3 and +1. The nucleotides between 0 and +5 are reciprocally exchanged between virus and host, and their exchange leads to detectable phenotypes in each organism: the excised host is defective in the gene that constains the secondary attachment site, and the excised virus chromosome is altered for reinsertion. Genetic analysis of the mutant excisants has allowed us to test and to reject two simple models of the role of the core (see summary) in virus-host recombination: 1) An increase in the homology between two cores does not lead to a commensurate increase in recombination frequency; and 2) The recombination frequency between a pair of cores is not simply related to the total deviation from the wild type nucleotide sequence. Thus the core is neither a simple homology element nor a simple recognition element. It may be a combination of the two.

2. Determination of the crossover point in a number of secondary attachment sites (including, most critically, the site described above) shows that all can be accounted for by assuming that there are only two possible crossover points in the core. This assumption is consistent

with the physical mapping of K. and M. Mizuuchi who find crossover points between core bases -3 and -1 on one polynucleotide strand and between core bases +3 and +5 on the complementary strand.

3. We are isolating, characterizing and sequencing mutants in the virus attachment site in order to determine which bases are important for function. We started with a 250 bp fragment that is the minimum that is fully functional in recombination. We have so far isolated and sequenced one ultraviolet light- and three bisulfite-induced mutations. Two of the latter have multiple base changes but have no defect in recombination. We surmise that many of the bases in the 250 bp attachment site are not absolutely required for efficient recombination.

4. It is known that the bacterial attachment site is considerably smaller than that of the phage, and it has been proposed that the 15 bp core region is sufficient for bacterial attachment site function. We have tested this idea by constructing and characterizing a novel attachment site that has a complete core, wild type bacterial DNA to the right of the core, and heterologous DNA to the left. This site did not have normal bacterial attachment site function and we conclude that specific sequences to the left of the core are also required.

5. We have previously described a new virus mutant, called xin (for xis independent), that appears to have an increased activity of integrase, a protein that promotes virus-host recombination. Increased integrase activity has several consequences: it increases the frequency of deletions, of phage insertion at secondary attachment sites, of insertion of a λ xin derivative that carries a partially defective attachment site (λb2 xin), and of recombination in the absence of active xis and hip proteins, two factors that stimulate virus-host recombination. The xin mutation does not appear to alter the specificity of integrase action as shown by the unchanged spectrum of secondary attachment site preference. Mapping of the xin mutation places it upstream of the coding sequences for integrase, and we suspect that it acts by increasing the transcription of the integrase gene or, less likely, the translation of integrase mRNA.

Proposed Course of Project:

We are attempting to cross the core mutations described in paragraph (1) above into the normal (primary) bacterial attachment site in order to determine their effect on function. In collaboration with T. Platt, we are sequencing another secondary attachment site in which imprecise excision occurs in the hope of characterizing a new core mutation. We are continuing to isolate, to sequence and to characterize mutations in the phage attachment. We are planning to sequence deletions induced by the xin mutation in order to confirm our assignment of cross-over loci described in paragraph (2) above. We are also planning to sequence the xin mutation itself.

Significance to Biomedical Research and the Program of the Institute: The insertion and excision of viral (and other episomal) DNA is known to change the physiology of the host organism. In addition, these viruses (and other episomal elements) provide an important mechanism of genetic transfer and exchange in the microbial world. Viruses promote genetic transfer because of their ability to convert host genes to an infectious form by packaging them inside a shell of viral structural proteins. It is highly likely that such a successful symbiotic relationship has an analogy in the relationship between certain animal viruses and the cells of higher organisms. In fact it is known that animal viruses can package the DNA of their hosts although the biological significance of this phenomenon is unknown. Moreover, an understanding of the control mechanisms operative in these easily studied microorganisms has obvious relevance to an understanding of regulatory elements in higher organisms. The ability to direct the insertion of phage DNA to pre-determined locations on the host chromosome is an extremely useful and widely applicable method for isolating gene products and studying the control of bacterial gene expression.

Publications:

1. Sternberg, N., Hamilton, D., Enquist, L. and Weisberg, R. A simple technique for the isolation of deletion mutants of phage lambda. Gene 8: 35-51, 1979.

2. Mizuuchi, K., Gellert, M., Weisberg, R. and Nash, H. Catenation and supercoiling in the products of in vitro λ integrative recombination. J. Mol. Biol., in press.

3. Enquist, L. and Weisberg, R. Lambda site specific recombination. Microbiology-1981, in press.

4. Nash, H., Mizuuchi, K., Enquist, L. and Weisberg, R. Strand exchange in λ integrative recombination: Genetics, biochemistry and models. Cold Spring Harbor Symp. Quant. Biol., in press.

5. Buraczynska, M., Enquist, L., Foeller, C., Hsu, P., Landy, A., Mizuuchi, K., Mizuuchi, M., Ross, W. and Weisberg, R. Structure and function of the phage λ attachment site: Size, int-protein binding, and location of the cross-over points. Cold Spring Harbor Symp. Quant. Biol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00067-12 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Integrative Control of Macromolecular Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Cashel	Medical Research Officer	LMG NICHD
	Yoshimi Ota	Visiting Fellow	LMG NICHD
	James Sylvester	Staff Fellow	LMG NICHD
	Sara Contente	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)
Dr. Geoffry Zubay, Columbia University, New York, New York

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project continues to be directed towards understanding how a cell coordinates the expression of its genetic repertoire during both normal growth and unbalanced nutritional deficiencies. Previous project work has described the discovery, characterization and regulatory attributes of a regulatory nucleotide (ppGpp) that is thought to participate in the regulation of about half of the genes of E. coli.

We discovered that ppGpp can protect the cell from otherwise lethal consequences of amino acids starvation as judged by the fact that mutants totally lacking ppGpp are killed by amino acid starvation. We have engineered a system for studying promoter structural determinants for regulation of gene expression. The S30 assay system for in vitro gene expression has been made about 100-fold more sensitive by genetically eliminating DNA exonuclease activity.

PHS-6040

Project Description:

Objectives: The contined aim of this project is to understand in molecular terms how cellular activities are coordinated. We are particularly concerned with regulation mediated by ppGpp. Our objectives are to understand how ppGpp is formed and how it acts.

Major Findings:

1. Biosynthesis of ppGpp. Previous reports have considered the synthesis of ppGpp mediated by the relA gene product functioning on ribosomes when messenger RNA codons bind to uncharged cognate tRNA. Mutants of the relA gene have always been capable of basal level synthesis of ppGpp which could accumulate during carbon or energy source starvation but not during amino acid starvation. We have characterized the physiological behavior of a mutant we isolated that is completely unable to synthesize ppGpp because of a mutation in another gene, relS, in addition to relA.

   During steady-state logarithmic growth there are no noticeable consequences of the presence or absence of basal levels of ppGpp. However when a relA, relS strain is amino acid starved cell death occurs with a few percent of cells surviving after 30 minutes. In contrast carbon source starvation is bacteriostatic rather than bacteriocidal. These comparisons demonstrate that ppGpp, like cAMP, has a regulatory and only a regulatory role in the cell. The phenomenon also provides the first means of revertant isolation.

2. relS-relA+ incompatibility. Cell strains bearing the relS mutation do not survive when the wild type relA+ gene is allowed to be expressed, but does survive in a relA missense mutant background or in a relA amber mutant under nonsuppressing conditions. This puzzling incompatibility has been demonstrated as a failure to recover certain expected recombinant classes in genetic crosses as well as during conditional suppression of relA amber mutants. We have looked for unusual nucleotide behavior under these conditions but have found no explanation for the lethality of this combination of genes.

3. Regulation of gene expression by ppGpp. We are simultaneously developing both in vivo and in vitro systems to measure regulation of gene expression by ppGpp. Previous reports have described cloning and characterization of an extremely active ribosomal RNA gene promoter. We have begun to insert portions of this complex region into a plasmid system developed by McKenney and Rosenberg to standardize measurements of promoter strengths. This plasmid has highly efficient transcriptional and translational terminators that can be used to stabilize inserts of extremely active promoters. We are developing this system so that a DNA fragment bearing a normal (or artificially modified) promoter can be easily transferred from a plasmid to a lambda transducing phage and back again. The biological activity of the DNA

fragment can then be measured in single copy lysogens and the fragment responsible for this activity recovered and verified.

4. This year we have discovered a means of enhancing the sensitivity of the standard "Zubay system S30" means of assessing gene expression in vitro. Assays of gene expression in these systems were difficult or even impossible using linear DNA fragments. We abolished most of the exonuclease from the extracts by introducing a recB allele into the strain otherwise optimized for extract preparation. This genetic modification of the strain resulted in approximately a 100-fold enhancement of its sensitivity. We are currently abolishing the major sources of RNase activity by further genetic manipulations and anticipate an even greater sensitivity. This in vitro system shows ppGpp inhibition of rRNA synthesis and now can be used for analogous studies with small, linear DNA fragments.

Significance to Biomedical Research and the Program of the Institute:
Integrative control processes are common to all growing and nutritionally adapting cells. This problem has turned towards a more basic understanding of how gene expression is initiated which will be essential for understanding primitive, differentiating and specialized cellular functions.

Proposed Course of Project:

We anticipate continued use of both genetic and biochemical approaches to understanding the ppGpp regulatory system. We will search for the cause of cell death when relA relS cells are amino acid starved especially from the point of view of unbalanced growth and possibly coding errors. We will try to understand why relA+ gene expression kills a relS strain as an additional clue to this system. We shall construct strains deficient in all known pleiotropic regulatory systems (ppGpp, cAMP and nitrogen starvation) in order to search for new systems. Finally we will continue our genetic dissection of structural determinants of ribosomal promoter activities.

Publications:

1. Engel, J.A., Sylvester, J. and Cashel, M.: Guanosine 3',5'-bispyrophosphate is a dispensable metabolite. In Koch, G. and Richter, D. (eds.): Regulation of Macromolecular Synthesis by Low Molecular Weight Mediators. New York, Academic Press, pp. 25-38, 1979.

2. Silverman, R., Atherly, A.G., Glaser, G. and Cashel, M.: Radioimmunoassay for ppGpp. In Koch, G. and Richter, D. (eds.). Regulation of Macromolecular Synthesis by Low Molecular Weight Mediators. New York, Academic Press, pp. 107-114, 1979.

3. Ota, Y., Kikuchi, A. and Cashel, M.: Gene expression of an E. coli ribosomal promoter fused to a galactose structural gene. Proc. Natl. Acad. Sci. U.S.A. 76, 5799-5803, 1979.

4. Yang, H.L., Ivashki, L., Hui-zha, C., Zubay, G. and Cashel, M.: A cell-free coupled transcription translation system for investigation of linear DNA segments. Proc. Natl. Acad. Sci. U.S.A., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00068-09 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Factors Influencing Genetic Transcription-Initiation and Termination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Crouch	Research Chemist	LMG NICHD
	Patricia E. Nelson	Staff Fellow	LMG NICHD
	Stephanie Seidman	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)
Margaret Stewart, LMG, NICHD; Dr. Philip Carl, University of Illinois

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

RNA plays an important role in cellular regulation--either by its presence in active form or by its total absence. A number of proteins have been shown to be involved in regulating RNA synthesis and RNA processing in Escherichia coli--among these are RNA polymerase, rho and RNase III. We have described an enzyme from chick embyros (RNase DII) which is an analogue of RNase III. These proteins are involved in the synthesis or inhibition of synthesis of specific species of RNA and in the processing of RNA. Another enzyme which may be involved in regulation of RNA synthesis is RNase H. The objective of this research project is to determine the method by which several of these enzymes act to regulate RNA synthesis in E. coli and chick embryos.

PHS-6040
(Rev. 10-76)

Objectives: Our goal has been to understand the control of synthesis and utilization of RNA transcripts at the enzymological level. Primary transcripts generally are cleaved to smaller products during the course of maturation. Such processing events have recently been shown to play a significant role in the formation of both messenger RNA and ribosomal RNA metabolism and has captured the interest of a number of scientists. We are studying an enzyme from chick embryos that was isolated in this laboratory and implicated in processing ribosomal RNA by studies on isolated nucleoli (the organelle in which synthesis and processing of ribosomal RNA is localized) and by the ability of this ribonuclease, RNase DII, to degrade double-stranded RNA to acid soluble-products--a property shared by one of the E. coli enzymes involved in ribosomal RNA processing, RNase III. Our attention is now focused on the sites of cleavage of ribosomal RNA precursors at the level of the precise nucleotide sequence that is cleaved.

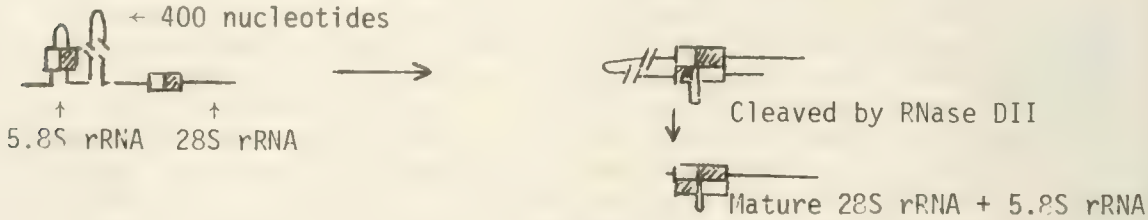
Methods Employed:

Experiments performed in this laboratory during the period of investigation covered by this report employed a variety of methods. Growth of bacteria containing recombinant DNA plasmids permitted the isolation of quantities of DNA to use restriction enzyme cleavage to generate fragments for DNA sequencing by the chemical method of Maxam and Gilbert. Chicken cells have been grown in tissue culture as a source of RNA. In many instances, cells have been fractionated into a number of subcellular components (e.g. nuclei and nucleoli) for isolation of RNAs peculiar to such fractions. RNA sequencing has been accomplished by first end labeling RNA with either γATP+ polynucleotide kinase (5' end label) or pCp+ RNA ligase (3' end label) then partial digestion with a variety of ribonucleases. Both restriction enzyme analyses of DNA and fractionation of RNA, as well as sequence analysis of both DNA and RNA, rely heavily on agarose and polyacrylamide gel techniques.

Major Findings:

1. Sequencing of one of the four processing sites has given us a great deal of insight into the structures involved in processing of ribosomal RNA. We have found that one part of the transcript, 5.8S rRNA, can exist, in the primary transcript, in a structure that is self-complementary or in a second state in which the 5.8S rRNA is hydrogen bonded to the region of the transcript corresponding to 28S rRNA. Either structure could be a site of cleavage for RNase DII. While the nucleotides involved in these alternative interactions are separated by some 600 nucleotides in the transcript, our sequence results show that these two regions are spatially closer since a large "hairpin" structure exists between them, bringing the two regions in close proximity.

2. Sequences which are adjacent to the processing sites suggest a model in which rearrangement of 5.8S rRNA occurs after an initial cleavage to form the 5' end of 5.8S rRNA. Mediation of the rearrangement may occur through U3 RNA and its associated proteins. U3 RNA is a small nucleolar RNA found in association with 32S rRNA (the immediate precursor to 28S rRNA + 5.8S rRNA).



3. In the course of our studies on 5.8S rRNA formation from ribosomal precursors in chickens, we have observed that, in vivo, two forms of 5.8S rRNA are found--one form having 5 or 6 nucleotides at its 5' terminus that are absent in the shorter form. The ratio of these two forms and the fact that the shorter form corresponds in sequence to 5.8S rRNAs from several sources suggests that both forms are in active ribosomes and that the differences reflect an alternative structure at the time of processing.

Proposed Course of the Project:

Two approaches to the problem of processing ribosomal RNA will be taken. Our previous results on RNase DII will be extended by making in vitro transcripts of certain cloned fragments containing ribosomal promoters of E. coli and various regions of the processing site(s) for the final cleavage of 32S rRNA to form 28S rRNA + 5.8S rRNA. Such transcripts will then be challenged with RNase DII and an examination of the products will permit us to assess the involvement of RNase DII in the process of ribosomal RNA maturation. Part of the model proposed from our sequence data suggests that a rearrangement of RNA structure is necessary for generating some of the appropriate cleavage sites. This part of the model can be tested by making in vitro transcripts that can form only one complex. Using various cloned fragments we should be able to make transcripts that contain only one of the two forms and are, therefore, limited to only one type of cleavage. Secondly, the model suggests the involvement of U3 RNA in the rearrangement. Evidence from other laboratories has implicated U3 RNA in processing but these studies have specified no mechanism. Our studies on U3 RNA will be important regardless of the exact mechanism involved. The study of such involvement of U3 RNA at this cleavage site will be assessed by 1) sequence analysis of U3 RNA in chickens, 2) study of the changes in U3 RNA under a series of conditions that alter the conversion of 32S rRNA to 28S rRNA (e.g. temperature sensitive mutants that are altered at this step, chemical agents that interfere with this process and various

viral infections which result in altered processing of ribosomal RNA) and 3) depending on the progress of work on items 1) and 2), combining U3 RNA (and any associated proteins) with RNase DII in the in vitro system and appropriate in vitro transcripts to mimic the proposed rearrangement of the ribosomal RNA transcript in vivo. These studies should permit us to determine 1) the involvement of RNase DII in processing ribosomal RNA, 2) the structural features necessary for correct processing and 3) the involvement of the small nuclear RNA (U3) in ribosomal processing.

Publications:

1. Stewart, M.L., Crouch, R.J. and Maizel, J.V., Jr. A high resolution oligonucleotide map generated by restriction of poliovirus type 1 genomic RNA by ribonuclease III. Virology, in press.

2. McClain, K., Stewart, M., Crouch, R.J., Sullivan, M., and Maizel, Jr., J.V.: Ribosome binding to polio virus RNA. 1980 Keystone Conference, in press.

3. Carl, P.L., Bloom, L. and Crouch, R.J.: Isolation and mapping of a mutation in Escherichia coli with altered levels of RNase H. J. Bacteriol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00069-08 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Molecular Aspects of the Replication of Enveloped Animal RNA Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith G. Levin	Research Biochemist	LMG NICHD
OTHERS:	Lesley I. Messer	Postdoctoral Fellow	LMG NICHD

COOPERATING UNITS (if any) Brenda Gerwin and Robert Bassin, Laboratory of Tumor Virus Genetics, NCI; Sisir Chattopadhyay, Pediatric Oncology Branch, NCI; Jonathan Seidman, LMG, NICHD; Anil Mukherjee, Pregnancy Research Branch, NICHD

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to define the molecular mechanisms involved in the replication of enveloped RNA viruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. The possible role of the viral RNA genome in directing virus assembly as well as certain aspects of viral RNA metabolism are being investigated in a system which uncouples murine leukemia virus RNA synthesis from terminal steps in virus maturation. The virus particles produced under these conditions resemble deletion mutants since they lack the 70S RNA genome and contain predominantly 4S RNA. Another major interest concerns the mechanism by which murine leukemia virus particles encapsidate a selected population of host tRNAs. Recent results indicate that the reverse transcriptase enzyme selects the tRNA primer used for viral RNA-directed DNA synthesis. Work is also being carried out on chromosome-mediated transfer of viral genetic information.

Project Description:

Objectives: To study factors which influence expression of viral genetic information; to define in molecular terms the specificity requirements of the virus assembly process; to understand the virus-host relationship in chronic infection.

Methods Employed: Mouse cells chronically infected with murine leukemia virus are maintained as continuous lines in culture. Leukemia virus production is measured by assay of the extracellular fluids for reverse transcriptase activity in response to synthetic templates. Infectivity is determined by the XC plaque assay. The RNA species present in virions are analyzed by polyacrylamide gel electrophoresis. Conditions have been devised which yield high resolution of a wide range of RNA molecules, from the high molecular weight 70S viral RNA genome to the low molecular weight 8S and 4S RNA species. Virus-specific RNA sequences are detected by hybridization with a viral cDNA probe. Viral proteins are analyzed on polyacrylamide slab gels. Electron microscopy is used to follow viral morphogenesis and to delineate the ultrastructural features of the virions.

Major Findings:

1. In earlier work on this project, a system was developed in which synthesis of murine leukemia virus RNA is uncoupled from terminal steps in virus maturation by treating chronically infected cells with actinomycin D. Despite the absence of ongoing viral RNA synthesis, virus particles continue to be assembled for at least 8-12 hours. Most of the virions produced are defective, however, and form a unique and previously undescribed class of RNA tumor virus particles which lack 70S genomic RNA and appear to contain only low molecular weight RNA species. We refer to these defective particles as Act D virions.

2. Studies on viral RNA.

   a. Viral RNA metabolism. The actinomycin D system is being exploited to study viral mRNA stability and pathways involved in utilization of viral RNA species for encapsidation or messenger function. Previously we showed that at 6-8 hours following actinomycin D treatment, viral mRNA function (as evidenced by continued viral protein synthesis) is maintained at 50% of the normal level, whereas viral infectivity and total amount of 70S RNA in the virion preparation are both reduced by more than 90%. These findings are now being extended. Intracellular and extracellular levels of viral RNA have been quantitated and compared in liquid hybridization experiments using a viral cDNA probe. Kinetic studies have revealed that over a period of 8 hours following addition of actinomycin D, the amount of virus-specific RNA sequences in virions is rapidly decreased and by 8 hours is 10% or less of the control level. In contrast, the level of intracellular virus-specific RNA sequences is reduced by only 2-fold up to 12 hours after actinomycin D treatment, in accord with the earlier data on viral protein

synthesis. By 24 hours, the decrease in intracellular viral RNA sequences is about 4-5 fold. Similar results were obtained with total cell RNA and polyA-selected cell RNA. These findings support our previous conclusion that viral messages have a longer half-life than RNA which is ultimately packaged into virions. Further work on this project is in progress.

b. Selective encapsidation of host tRNAs. PNA tumor viruses contain a selected population of host tRNA molecules. Since the reverse transcriptase enzyme is known to interact with tRNAs which serve as primers for RNA-directed DNA synthesis, we have been studying the involvement of reverse transcriptase in the tRNA selection process. Studies were carried out with two classes of nonconditional virus mutants. One mutant, clone 23, synthesizes an altered polymerase molecule. Upon repeated analysis, we now find that this mutant has the same tRNA composition as wild-type virus. Furthermore, the levels of proline tRNA primer in both free 4S RNA and genome-associated 4S RNA are normal. Thus, the tRNA primer binding site on the clone 23 polymerase appears to be unaffected by the mutation. In contrast, previous studies with a mutant lacking reverse transcriptase, M13, and more recently with a related mutant, M13/1, have shown that the free 4S RNA in these virions is markedly deficient in proline tRNA. This deficiency is not absolute, however, and we detect normal amounts of proline tRNA associated with the 35S RNA genome. These results indicate that i) during virus assembly, the reverse transcriptase enzyme plays an important role in the selection of the viral tRNA primer; and ii) since the tRNA primer is found associated with 35S RNA in the absence of enzyme, reverse transcriptase is not required to mediate conformational changes in the primer structure which are a prerequisite for tRNA binding to genomic RNA.

3. Interest in <u>in vitro</u> methods of gene transfer has led to the development of a system in which chromosomes from murine leukemia virus-infected cells can be used to transfect other cells. Control experiments have ruled out the presence of intact virus particles or cells in the chromosome preparations. Chromosomes or chromosomes contained in liposomes (lipochromosomes) are equally effective. Virions produced as a result of chromosome-mediated transfection are infectious and have the same biological characteristics as virus produced by the donor cells from which the chromosomes are isolated. An advantage of chromosome-mediated transfection is the ability to cross the normal species barrier to virus infection. Thus, although murine leukemia virus particles normally infect only mouse cells, using chromosomes from AKR virus-infected mouse cells, we have been able to productively infect heterologous mink cells.

Signficance to Biomedical Research and the Program of the Institute: The advantages of viral systems as models for studying the regulation and expression of genetic information are well recognized. Studies on the RNA tumor viruses are of special interest since these viruses establish a chronic infection in the host cell by integrating viral genetic information into the host chromosome. An understanding of the various steps involved in tumor virus replication and mechanisms for generating defective virions should aid in efforts to limit the oncogenic potential of these agents. Finally, information on the degree to which host material can be assembled into virus particles could have some application in the field of gene therapy, where the use of pseudotype virions has been contemplated as a means of introducing "foreign" nucleic acid into an individual possessing an inherited biochemical defect.

Proposed Course of the Project:

1. Studies on viral RNA metabolism will be continued with the actinomycin D system. Interest is now focused on determining whether the intracellular viral RNA sequences which appear to be so stable in the absence of viral RNA synthesis in fact represent intact viral mRNA species. In order to analyze viral RNA, which is less than 1% of total cell RNA, very sensitive detection methods must be employed. We are now in the process of setting up a denaturing gel electrophoresis system and the "Northern" blotting procedure. A nick-translated cloned viral DNA probe will be used for the hybridization. Experiments are also being carried out to characterize the extracellular RNA sequences present in the Act D virion preparation.

2. Work will continue on the chromosome system. We are interested in determining the efficiency of chromosome-mediated transfection. It is not possible to do this in experiments with chromosomes from murine leukemia virus-infected cells since virus production in recipient cells can only be detected after virus spread has occurred. To approach this question, we will take chromosomes from a murine sarcoma virus-infected nonproducer cell line and determine whether uninfected cells become transformed, as evidenced by induction of foci. In this situation, each focus would originate from successful infection of a single cell and since no particles are produced, values for specific activity can be estimated. Preliminary results suggest that such experiments are feasible. We also plan to see whether viral genetic information is integrated into high molecular weight DNA of recipient cells following transfection. This will be tested by analysis with appropriate restriction enzymes and cloned viral DNA probes.

3. Analysis of viral mutants has been useful in studying specific aspects of viral replication. The availability of clone 23, a nonconditional mutant in reverse transcriptase, provides an opportunity to learn more about the viral mRNA which codes for polymerase and about the protein product which is generated as a result of the genetic lesion. Further characterization of this mutant is therefore contemplated. Although we have previously isolated the 70S RNA complex from virions, we will now examine the subunit size more carefully in denaturing gels. We also plan to characterize intracellular mRNA species, using the Northern blot procedure. In vitro translation with virion RNA as template and if possible, with genome-size mRNA will be carried out. Plans to map the genetic defect in clone 23 by restriction enzyme digestion are contingent upon results of control experiments now in progress to determine whether endogenous viruses present in the cells will interfere with the analysis.

Publications:

1. Gerwin, B.I., Rein, A., Levin, J.G., Bassin, R.H., Benjers, B.M., Kashmiri, S.V.S., Hopkins, D. and O'Neill, B.J. Mutant of B-tropic murine leukemia virus synthesizing an altered polymerase molecule. J. Virol. 31: 741-751, 1979.

2. Bassin, R.H., Gerwin, B.I., Levin, J.G., Duran-Troise, G., Benjers, B.M. and Rein, A. Macromolecular requirements for abrogation of Fv-1 restriction by murine leukemia viruses. J. Virol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00070-20 LMG

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Morphogenesis of Animal Viruses During Infection of Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob V. Maizel, Jr.	Research Chemist	LMG NICHD
OTHERS:	Margaret Stewart	Chemist	LMG NICHD
	Robert P. Lenk	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)

Dr. N. Salzman, LBV, NIAID; Dr. L. Lipkins, DCBC, NCI; Dr. G. vande Woude, VB, NCI; Dr. B. DeCrombrugghe, NCI; Dr. Marc Girard, Pasteur Institute, Paris

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mammalian virus systems have been studied by combined electron microscopic and biochemical methods. Oligonucleotides and ribosome binding sites of the poliovirus genome have been mapped. Polio RNA fragments have been translated in vitro, and cloned in plasmids. Adenovirus early proteins have been localized in the cellular ultrastructure. Newly devised computer programs have been used to analyze nucleics for homologies, secondary structures, splice sites, promoter sites and recombination sites.

PHS-6040

Project Description:

Objectives: Our objective is to describe in molecular detail the components of animal viruses and infected human cells with the aim of thereby pinpointing the molecules that cause genetic and non-genetic changes in transformation and infection. The animal virus systems used as models have been poliovirus, adenovirus and murine oncornaviruses.

Methods Employed: Each virus system has been examined with the techniques of isotopic tracer analysis in vivo and SDS gel electrophoresis to detect all of the viral proteins and nucleic acids as they are newly synthesized and modified and to detect changes in cellular proteins. In combination with subcellular fractionation techniques individual proteins can be followed through their synthesis and assembly into multimeric forms, and localization in subcellular fractions. By such fractionation powerful clues as to the structural and functional roles are obtained. Electron microscopy is used to characterize individual and complex macromolecules and to localize biologically active molecules through the use of radioautography and immuno-electron microscopy. Computer analysis is used to reveal important structural features in nucleic acid sequences.

Major Findings:

1. Poliovirus. The map of poliovirus genomic fragments by analysis of 76 oligonucleotides from RNAse III fragments has been completed. A remarkable outcome of this work was the realization that the major sites of cleavage by RNAse III, an enzyme that cleaves single-stranded RNA at positions where secondary structures occur, coincide very well with the boundaries of mature viral proteins. In concurrent studies in this Section is was observed that at least three sites for binding of eukaryotic ribosomes could be detected by attachment and protection of otherwise susceptible 30-40 base oligonucleotides to nuclease digestion, and by observation in the electron microscope of bound and chemically fixed ribosomes on poliovirus RNA molecules. All of the above evidence suggests that there may be some unanticipated intricacies built into the polio genome. The general dogma is that all of polio is translated into a single polyprotein, which then is cleaved into a series of final proteins that we and others have described before. Now we must consider that the genome has points of recognition for enzymes and ribosomes that could allow alternate mechanisms of expression. It is conceivable that secondary, internal sites of ribosomal initiation of translation can occur. This theory violates a dogma of eukaryotic translation which states that initiation starts at the AUG codon nearest to the 5' end of mRNA. A unifying view could arise from the idea that eukaryotic RNAse III-like enzymes cleave the genomic message at sites which allow cryptic ribosome recognition sites to function. An observation of considerable significance is that a 3000 base genomic RNAse III fragment from the 3' end of the chromosome

in vitro is translatable into a polypeptide physically similar to the mature polymerase polypeptide of MW 56000.

Work has begun on the production of poliovirus cDNA clones in plasmids. In collaboration with Dr. Marc Girard, characterization of some clones has begun by oligonucleotide hybridization and by electron microscopy of polio RNA/clone DNA and and heteroduplex structures.

2. Adenovirus. The mapping of early 19K glycoproteins has been completed, and a number of laboratories have confirmed that it is encoded in early region 3 (map position 76.7-86.0) as noted in last year's report. This protein had earlier been observed by us in biochemical preparations of plasma membrane. Its function is obscure. It seems not to be essential since non-defective adeno-SV40 hybrids lack it but nevertheless can grow.

   In analyzing preparations of cytoskeletons from adenovirus-infected cells we have noted that; among others, 19K protein remains attached. This demonstrates it as a type of protein that probably has a role in contact between the plasma membrane and the cytoskeletion.

   In an effort to test the functional effects of early proteins and to identify the protein or proteins responsible for cellular ultrastructural changes techniques for cell and lipid vessicle fusion have been set up and hybridomas against adeno proteins are being produced. These techniques will be used to induce viral modifications by introduction of early proteins and to block changes with suitable anti-early viral agents.

3. Methdology. Computer analysis of nucleic acid sequences has continued with refinement of the programs to produce graphic matrix analyses and application of the method to a variety of sequences in collaboration with a number of units. A version has been produced which can produce compressed scale plots so that large sequences an be analyzed on a single plot to reveal regions of interest for detailed analyses and for publication. The largest sequence analyzed thus far is 12000 bases representing the left 25% of bacteriophage T7. Considerable encouraging success has been achieved in implementing a type of search based on consensus sequences using a compliant matching procedure. This approach has been applied to sequences for *E. coli* RNA polymerase promoters using the consensus rules of Siebenlist *et al.*, eukaryotic intervening sequence splice signals using Seif, Khouri and Dhar's rules, and for limited work on attachment sites for lambda phage integrative recombination using data of Weisberg. At this stage the programs have not been extensively tested in a predictive mode, but are relatively successful at detecting known sites. As an example, it is possible to find all seven of the known *E. coli* RNA polymerase promoter sites of fd phage with only four

additional sites. It is even possible that the four extra sites are relevant but that data is merely not available. In a similar way the SPLICE program finds all the known splices in mouse beta globin gene sequences and a few more in the large intervening sequences that could be used in sequential splicing reactions that have been suggested to occur. An intermediate was predicted to occur from collagen gene sequence data at the same time as it was observed in the laboratory of de Crombrugghe et al.

Work has begun with Dr. Bruce Shapiro of the NCI to utilize their image analysis facility to develop an interactive graphic matrix program with the stability energy of reverse complements displayed and an interactive light pen to allow selection of favorable pathways to assemble into secondary structures. The goal is to allow larger sequences to be built into stable structures than can be done by the present combinatoric analyses. The latter methods become prohibitively expensive and unreliable with sequences of more than a 200-300 bases. These programs will then be used to predict structures for RNA molecules that can be observed in the electron microscope. Interaction of the previously devised structure mapping programs and predictions from sequences will aid developing electron microscope methods for RNA secondary structure analysis.

4. Collaborations. An important activity of this Section has continued to be collaboration with other units of the Laboratory and elsewhere. With a small Section it has been possible to apply the techniques to a larger number of important problems than otherwise possible. Electron microscopy and computer analyses have been applied to cloned retrovirus sequences from vande Woude's and Paston's sections, to cloned collagen with de Crombrugghe's section, and cloned poliovirus cDNA fragments with Girard (Paris).

Proposed Course of Project:

We will continue to develop electron microscope, biochemical and computer techniques to characterize molecules and relate them to the structure and workings of cells. Efforts will be continued to devise similar EM tags for molecules and to use them in mapping molecules and cells. Work will continue on ways to reveal information contained in nucleic acid sequences. We will explore ways to use information from nucleic acid sequences to alter the replication of viruses within cells.

Significance to Biomedical Research and the Program of the Institute:
Biochemical characterization of the early events in viral infections has more completely defined the critical events in cellular and viral regulatory mechanisms. New techniques using electron microscopy are now permitting visualization of molecules in the process of carrying out their regulatory roles. Observations of the specific interactions between cellular and viral gneomes and their transcriptional and translational products strikingly

verify and extend biochemical findings. Information thus derived will find application in control of reproduction, growth, differentiation and viral diseases in higher organisms. Beginnings to the design of "magic bullets" to selectively affect cellular or viral proteins at the level of genomic expression are being realized.

Publications:

1. Lundquist, R.E., Sullivan, M., and Maizel, Jr., J.V.: Characterization of a new isolate of poliovirus defective interfering particles. Cell 18, 759-769, 1979.

2. Konkel, D., Maizel, J. and Leder, P.: The evolution and sequence comparison of two recently divergent mouse chromosomal β-globin genes. Cell 18, 865-873, 1979.

3. Shapiro, B., Lipkin, L., and Maizel, Jr., J.V.: Computerized generation of secondary structure maps for nucleic acids. Computers in Biomedical Research 12, 615-630, 1979.

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10. Oskarsson, M., McClements, W., Blair, D.G., Maizel, J.V., and vande Woude, G.F.: Properties of a normal mouse cell DNA sequence (sarc) homologous to the src sequence of Moloney sarcoma virus Science 207, 1222-1224, 1980.

11. Sherr, C.J., Fedeles, L.A., Oskarsson, M., Maizel, Jr., and vande Woude, G.F.: Molecular cloning of Snyder-Theilen feline leukemia and sarcoma viruses: comparative studies of feline sarcoma virus with its natural helper and with Moloney sarcoma virus. J. Virol. 241, 200-212, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 HD 00071-08 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Study of Adenovirus Gene Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Westphal	Research Geneticist	LMG NICHD
	G. Kitchingman	Research Associate	LMG NICHD
	W. Richardson	Visiting Fellow	LMG NICHD

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Animal Viruses

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is the analysis of the elements that control adenovirus gene expression in lytically infected or in transformed cell cultures. Topics currently under investigation include: (a) the nature of early adenovirus functions that promote the growth of defective adeno-associated viruses, (B) molecular events that determine the host range of the hybrid virus Ad2+ND4 and (C) generation of adenovirus mutants which carry lesions in individual early gene regions. Besides standard procedures of molecular genetics and biochemistry, techniques such as micro-injection of animal cells, electron microscopy and gene cloning in prokaryotic vectors, are used in these studies.

PHS-6040

Project Description:

Objectives: Our group studies the elements that control viral gene expression in cultured animal cells.

Methods Employed: See Summary.

Major Findings:

A. Extensive mapping of early nuclear and cytoplasmic adenovirus type 2 (Ad2) RNA in the electron microscope has revealed a complex pattern of RNA processing resulting in sets of distinct mRNAs corresponding to each of the four major early regions of the viral genome. Additional regions of the genome have been found to give rise to rare forms of early Ad2 RNA and thus to contribute to early Ad2 gene expression.

B. Spliced forms of mRNA were detected and mapped for adenovirus-associated virus (AAV). Like adenovirus RNAs, the AAV mRNAs appear to be derived from unspliced precursor molecules.

C. Using the microinjection technique, early Ad2 mRNA has been defined as necessary and sufficient to promote the growth of AAV.

D. The structure of SV40 DNA inserted in the genome of the nondefective hybrid virus Ad2+ND4 is closely correlated with the host range of this virus. About 500 base pairs of SV40 DNA including the early splice signals, are removed when the hybrid virus is propagated in human cells. The 500 base pair DNA segment is required for growth in monkey cells, however.

Significance to Biomedical Research and the Program of the Institute: Our studies are designed to contribute to an understanding of the molecular events which accompany cell infection by a virus that is known as a human pathogen in childhood diseases, as well as a tumor virus in rodents. As shown above, our experiments offer insights into basic mechanisms of genetic control and regulation in eukaryotic cells.

Proposed Course of the Project: (a) Characterization of individual Ad2 gene functions responsible for AAV growth. Individual species of early Ad2 mRNA will be purified by hybridization to cloned segments of Ad2 DNA. Purified mRNAs will be micro-injected into cells infected with AAV. (b) Early adenovirus mutants. Established gene transfer techniques will be employed in an effort to insert individual early gene regions of Ad2 in cells permissive for Ad2 growth. Cells expressing the inserted genes will be used to isolate host range mutants carrying lesions in one or the other of the early regions. Attempts will also be made to introduce lesions into early genes by site specific mutagenesis. Early viral mutants are urgently needed for the elucidation of the biological functions of individual early Ad2 genes. (c) The DNA modification of Ad2+ND4. In continuation of

our current studies, we will try to establish the molecular basis for the observed, host-dependent DNA modification.

Publications:

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2. Richardson, W.D., Carter, B.J. and Westphal, H. Vero cells injected with adenovirus type 2 mRNA produce authentic viral polypeptide patterns. Early mRNA promotes growth of adenovirus-associated virus. Proc. Natl. Acad. Sci. U.S.A. 77: 931-935, 1980.

3. Laughlin, C.A., Westphal, H. and Carter, B.J. Spliced adenovirus associated virus RNA. Proc. Natl. Acad. Sci. U.S.A. 76: 5567-5571, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00072-13 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Crystallin Synthesis in Development and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joram Piatigorsky	Biologist	LMG NICHD
OTHERS:	Toshimichi Shinohara	Staff Fellow	LMG NICHD
	Raymond E. Jones	Staff Fellow	LMG NICHD
	Suraj P. Bhat	Visiting Fellow	LMG NICHD
	Harry Ostrer	Clinical Associate	LMG NICHD
	George Inana	Research Associate	LMG NICHD

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Cellular Differentiation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.1	5.0	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Emphasis has been placed on characterizing crystallin genes. Two δ-crystallin genes with 14 to 15 intervening sequences have been identified and analyzed in genomic clones derived from chickens. Clones of α-, β- and γ-crystallin cDNAs have been obtained from mice and are being characterized in order to study the corresponding genes in this organism. One β-crystallin polypeptide has been shown to be synthesized exclusively in elongating lens cells suggesting differential expression of the β-crystallin genes during fiber cell differentiation. Spectroscopy studies have demonstrated age-dependent changes in the conformation of δ-crystallin in chickens. Experiments with virally-infected lens epithelial cells have dissociated changes in cell growth and morphology from δ-crystallin gene expression. Finally, the initial reduction of crystallin synthesis in two hereditary cataracts in mice has been shown to be associated with poor utilization of persistant crystallin mRNAs due, at least in part, to an increase in K+ and decrease in Na+ within the lens.

Objectives: To understand the regulation of protein (crystallin) synthesis in clear and cataractous lenses, particularly at the gene level during development.

Methods Employed: Conventional techniques of nucleic acid and protein biochemistry, as well as recombinant DNA methods, are applied.

Major Findings:

1. Significant advances have been made towards characterizing the crystallin genes. Two δ-crystallin genes with 14 to 15 intervening sequences have been identified in chickens, suggesting that the synthesis of δ-crystallin may be directed by two mRNAs transcribed from different genes. Analysis of cloned fragments of each gene showed that the mRNA gene sequences are very similar while 60% divergence was found between the intervening sequences of the two genes. Insertions and/or deletions appear to be major events responsible for these differences.

2. cDNA clones of α, β- and γ-crystallin of the mouse have been obtained and are presently being characterized.

3. The synthesis of β-crystallin polypeptides has been shown to be differentially regulated within the developing chick lens. The largest (35,000 daltons) of seven β-crystallin subunits is synthesized only in elongating lens epithelial cells and maturing lens fiber cells. The cuboidal lens epithelial cells lack the mRNA for this β-crystallin polypeptide.

4. Circular dichroism and fluorescence spectroscopy have indicated considerable conservation of the conformation of δ-crystallin throughout evolution. Interestingly, appreciable age-dependent differences were noted in the conformation and fluorescence intensity of chicken δ-crystallin.

5. Primary cultures of embryonic chick lens epithelial cells have been transformed with a temperature-sensitive Rous sarcoma virus. The transformed cells adopted a fibroblastic appearance and severely reduced both δ-crystallin synthesis and δ-crystallin mRNA content. The virally-infected cells reversed to an epitheloid morphology at the non-permissive temperature for the src gene but did not initiate δ-crystallin synthesis or accumulate δ-crystallin mRNA.

6. Lens fiber cells of two hereditary cataracts in mice (Nakano and Philly) were shown to possess a full complement of crystallin mRNAs despite severely reduced crystallin synthesis in these cells. Crystallin synthesis was stimulated in the fiber cells by raising the K+ and lowering the Na+ concentration in the cultured lenses. The findings indicate that the reduction in crystallin synthesis in the initial stages of cataractogenesis in these lenses is due to poor utilization of crystallin mRNAs.

Significance to Biomedical Research and the Program of the Institute: These studies contribute to our knowledge of the mechanisms of cellular differentiation and disease, especially with regard to the mechanisms of specific protein synthesis in developing and cataractous lenses.

Proposed Course of the Project: The cloned δ-crystallin gene sequences will be analyzed further with particular attention given to promoter sites. The mouse α-, β- and γ-crystallin cDNA clones will be characterized in greater detail and used to isolate the corresponding genes and to quantify the corresponding mRNAs in clear and cataractous lenses in this species; attempts will be made to utilize these cDNA clones as probes in other species by cross-hybridization. The regulation of β-crystallin synthesis will be explored in the chicken lens; these experiments will involve cloning chicken β-crystallin cDNAs. Evolutionary studies on δ-crystallin will be continued.

Publications:

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2. Shinohara, T., and Piatigorsky, J.: Age-dependent differences in the ratio of synthesis of δ-crystallin polypeptides in cultured vitreous-free lenses of chick embryos. Develop. Biol. 72, 385-389, 1979.

3. Bhat, S.P. and Piatigorsky, J.: Molecular cloning and partial characterization of δ-crystallin cDNA sequences in a bacterial plasmid. Proc. Natl. Acad. Sci. U.S.A. 76, 3299-3303, 1979.

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7. Kador, P., Uga, S. and Piatigorsky, J.: The Philly mouse hereditary cataract. In Ageing of the Lens (ed. F. Regnault, O. Hockwin and Y. Courtois) pp. 157-170, Elsevier/North Holland Biomedical Press, 1980.

8. Bhat, S.P., Jones, R.E., Sullivan, M.A. and Piatigorsky, J.: Chicken lens crystallin DNA sequences show at least two δ-crystallin genes. Nature 284, 234-238, 1980.

9. Shinohara, T. and Piatigorsky, J.: Anion and cation effects on δ-crystallin synthesis in the cultured embryonic chick lens and in a reticulocyte lysate. Exp. Eye Res. 30, 351-360, 1980.

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13. Jones, R.E., DeFeo, D. and Piatigorsky, J.: Initial studies on cultured embryonic chick lens epithelial cells infected with a temperature-sensitive Rous sarcoma virus. Vision Res., in press.

14. Horwitz, J. and Piatigorsky, J.: Evolutionary and developmental differences in delta-crystallin from bird and reptile lenses: Circular dichroism and fluorescence studies. Biochem. Biophys. Acta, in press.

15. Piatigorsky, J., Shinohara, T., Bhat, S.P. and Jones, R.E.: Changes in crystallin synthesis in cataracts: A molecular approach. In The Red Blood Cell and Lens Metabolism (ed. S.K. Srivastava), Elsevier Noth-Holland Publishing Co., in press.

16. Ostrer, H., and Piatigorsky, J.: β-Crystallins of the adult chicken lens: Relatedness of the polypeptides and their aggregates. Exp. Eye Res., in press.

17. Jones, R.E., Bhat, S.P., Shinohara, T., and Piatigorsky, J.: New directions for cataract research: Use of recombinant DNA technology. In New Directions in Ophthalmic Research (ed. M. Sears) Yale University Press, in press.

18. Shinohara, T. and Piatigorsky, J.: Persistance of crystallin mRNAs with reduced translation in hereditary cataracts in mice. Science, in press.

19. Jones, R.E., Bhat, S.P., Sullivan, M.A., and Piatigorsky, J.: Comparison between two δ-crystallin genes in the chicken. Proc. Natl. Acad. Sci. U.S.A., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00073-09 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of Antibody Response at the Cellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edgar E. Hanna	Microbiologist	LMG NICHD
OTHER:	Michael L. Misfeldt	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)

C.T. Hansen, Geneticist, VR, DRS, NIH

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	2.0	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experiments are directed toward understanding processes by which regulation of the immune system is controlled at the cellular level. The integrated roles of regulatory cells such as thymus-derived helper and suppressor T-cells are being defined. We are seeking to understand T-cell circuitry from the precursor stage through the various phenotypic stages between suppressor and helper functional stages. Our results involving the preferential inactivation of suppressor function of T-cells by natural microbial agents such as the streptococal exotoxin, SPE, have delineated a novel approach towards understanding regulatory pathways and T-cell circuitry in the immune system. We have recently achieved stabilization of the helper T-cell phenotype as a functional T-cell hybridoma. Others being cloned appear to have other important activities. This approach is facilitating the ascertainment of definitive regulatory roles of T-cells. These monoclonal and functional T-cell clones also provide stable cellular targets for understanding the mechanisms of interference of microbes and their products in the immune system.

Project Description:

Objectives: To understand the processes by which regulation of the immune system is controlled at the cellular level. Specifically, we seek to understand the mechanisms by which thymus-derived T-cells regulate antibody formation and secretion by interaction with bone marrow derived precursors (B-cells) of antibody-forming cells. We seek to understand the interrelationships between the various T-cell phenotypes, from the precursor stage progressively through those having suppressive activity, to those expressing helper activity. We also seek to understand the origins and mechanisms of those T-cells which have a native effector function. We hope to derive information on the relationship of such effector T-cells and the regulatory T-cells. It remains unclear whether helper cells and suppressor cells are distinct cell types or the same cell type expressing different phenotypes in response to different stimuli, or whether such different phenotypes may reflect different stages of development and maturation. We seek to obtain clones of T-cells involved so as to determine the molecular nature of the T-cell receptor(s). Monoclonal lines of T-cells will also allow a better understanding of the receptors involved in binding of microbial and plant agents such as SPE and other mitogens, thus supporting modulation of the immune system. Our progress in constructing monoclonal lines of functional T-cells will support the isolation of the genes coding the various T-cell receptors.

Methods Employed: Major advantage is being taken of a library of NFR/N mouse T-cell hybridomas which we have recently constructed. The cellular reconstruction system (cellular complementation in vitro) which has been a major advantage to the progress of our experiments will facilitate the testing of our T-cell hybridomas for function. We anticipate construction of monoclonal cell lines that represent other major classes of immunocytes. We will continue to use nude mice as our source of T-cell deficient immunocytes. Nude mouse immunocytes will also be the cell source for the construction of B-cell and macrophage monoclonal lines. Type II, nude mice were the source of presumptive T-cell precursors from which we have also constructed hybridomas. NFR-nu/nu mice are now highly inbred to greater than 99.9% homogeneity. Standard immunological methodology has been detailed in previous reports.

Major Findings:

We have succeeded in constructing several hybridoma cell lines from fractionated T-cells from normal NFR/N mice. We have also constructed several hybridomas within fractionated nylon wool excluded NFR/N nude mouse splenocytes. These are a source presumptively of T-cell precursors. These were all constructed by the fusion of the respective fractionated splenocytes with malignant cells of the AKR mouse thymus lymphoma line BW5147. We have established a large library reserve of these hybridoma cell lines. We have cloned a small number of these lines so far representing Pool I (helper) and Pool II (suppressor) cells. We have tested three of the Pool I hybridoma

lines for activity and for T-cell surface markers. All three are hyperploid lines. Two of them tested so far express the Thy 1.2 T-cell marker of their NFR/N parent cells. The AKR parent cells express the Thy 1.1 marker. These hybrids express the Lyt-1 marker characteristic of helper T-cells like their Pool I T-cell parents. Both hybrids express the Fc and V_H T-cell surface markers. One of the two hybridomas has a high capacity to express a helper T-cell function and the other expresses no helper function as assessed in our cell complementation assay in vitro. Other hybrids although not yet characterized appear to represent a gradation of T-cell phenotypes.

Proposed Course of Project:

We will screen our library of hybridomas for activities representing the major activities which have been ascribed to regulatory T-cells. Those expressing interesting activity profiles will be cloned and characterized on the basis of the battery of T-cell surface marker tests. We have begun to prepare alloantisera against the hybrid lines by immunization of NFR/N mice and AKR/N mice with selected hybrid clones. These antisera are expected to provide standard antisera which should facilitate screening of subsequent monoclonally produced antisera in a related project designed to classify the NFR/N mouse and our hybrid lines for allotype and idiotype. We expect as our work progresses to define a gradation of T-cell phenotypes within our library.

Significance to Biomedical Research and the Program of the Institute:
Understanding the natural mechanism of the regulated expressions of the immune system may reveal pathways by which defective immune systems may be ameliorated. The potential ability to deregulate and to re-organize the immune system is highly relevant to amelioration of disease. Our demonstration that a representative microbial agent such as SPE can deregulate the immune system is relevant to an understanding of mechanisms of disease processes. Moreoever, results of our study should complement scientific thought on cellular control mechanisms in general.

Publications:

1. Hale, M.L. and Hanna, E.E. Complementation of the anti-TNP PFC response of N:NIH nude mice from nude dams by spleen cells of nude mice from heterozygous-for-nude dams. Cellular Immunology 47: 429-436, 1979.

2. Hanna, E.E. Hybridomas: Fusion of lymphocytes and mouse plasmacytomas--an approach to specific monoclonal antibodies. In D. Schlessinger (ed.), Microbiology-1980, American Society for Microbiology, Washington, D.C., 1980, in press.

3. Hanna, E.E., Hale, M.L. and Misfeldt, M.L. Deregulation of T-cell dependent PFC responses of mouse immunocytes is a common property of highly purified and of crude preparations of streptoccocal pyrogenic exotoxin. Cellular Immunology, in press.

4. Misfeldt, M.L. and Hanna, E.E. Fractionation of a T-cell subpopulation which generates SPE-induced deregulation of PFC responses. Cellular Immunology, in press.

5. Hanna, E.E. and Misfeldt, M.L. ANTIBODY-Monoclonal Antibodies (Lymphocytic hybridoma), Method and Application. In The 1981 McGraw-Hill Yearbook of Science and Technology, in press.

6. Hanna, E.E. and Misfeldt, M.L. Modulation of suppressor T-cells by Streptoccocal Pyrogenic Exotoxin. In, Spector, S., Friedman, H., and Szentivanyi, A. (eds.) Immunomodulation by Bacteria and Their Products, University Park Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00074-09 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Regulated Gene Expression During Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Leder	Chief	LMG NICHD
OTHERS:	N. Hansen	IPA	LMG NICHD
	D. Konkel	Staff Fellow	LMG NICHD
	I. Kirsch	Research Associate	LMG NICHD
	E. Max	Research Associate	LMG NICHD
	Y. Nishioka	Visiting Fellow	LMG NICHD
	J. Ravetch	Research Associate	LMG NICHD
	C. Talkington	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)
D. Hamer, NIAID, NIH; S.-P. Kwan and M. Scharff, Albert Einstein College of Medicine

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
11	8.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are attempting to understand the regulated expression of the globulin and immunoglobulin genes. For this purpose we have cloned and determined the structure of and a physical map for both the mouse alpha and beta globin genes as well as the mouse kappa immunoglobulin genes. In so doing we have identified sites critical to the transcription, polyA addition and splicing of the mRNA precursors using both in vivo and in vitro assays. We have also discovered and determined the sequence of several globin pseudogenes, including one entirely lacking intervening sequences. We have also shown how immunoglobulin gene diversity must arise and tested various models to account for site specific immunoglobulin gene recombination and its role in generating immunoglobulin diversity.

PHS-6040

Project Description:

Objectives: Our objective is to understand, in molecular terms, the mechanisms which regulate the flow and exchange of genetic information during cell growth and development. We use two model systems, one involving the process of erythrodifferentiation, the other, immunodifferentiation. Specifically we ask how genetic elements corresponding to globin and immunoglobulin are organized in the genome, how they arise during evolution, what elements in the genome and what gene products regulate their expression and how this regulation and organization are relevant to other elements in the genome of higher organisms.

Methods Employed: These investigations have, naturally, involved an interdisciplinary approach. In addition to the usual techniques of cell growth and culture and the biochemistry of nucleic acids and proteins, special attention has been given to the development of specific techniques for the manipulation of recombinant DNA molecules and their detection.

Major Findings:

1. The complete nucleotide sequence of the adult mouse alpha and beta globin genes and portions of their flanking sequences have been determined. A comparison of these has allowed us to identify specific segments that might be involved as transcription promoters or polyA addition signals.

2. The function of specific segments has been tested by introducing portions of both alpha and beta globin genes into the simian virus SV40. Using these hybrid viruses to infect cultured cells, we have found that at least one intervening sequence (gene interruption) is required for the expression of these genes. We have also located transcription promoter activity on the 5' side of the mouse alpha gene.

3. We have adapted and modified a simple cell-free system that accurately initiates transcription of globin (and other genes) and shown that the alpha globin promoter is located within a 150 base pair long sequence of DNA. Using segments derived from mutant genes (see below) we have been able to specify nucleotide positions that are likely to be important in specifying promoter activity.

4. We have isolated and completely sequenced an unusual alpha-like globin gene that entirely lacks intervening sequences and devised a model to account for the mechanism by which this structure arose.

5. We have expanded and tested our model for the formation of active immunoglobulin genes by showing that V/J recombination is accompanied by the deletion of both V and J region segments.

6. We have accounted for a major source of immunoglobulin diversity by showing that the crossover point of V/J recombination can vary so as to produce diversity at a critical region of the polypeptide chain.

Significance to Biomedical Research and the Program of the Institute:
These studies are directed towards understanding fundamental processes involved in the expression of genetic information. They are, thus, obviously relevant to achieving an understanding of a variety of inherited disorders that afflict man. By inherited disorders we refer not only to clearly identifiable conditions which involve mutations at a single genetic site, but also polygenic disorders such as diabetes, hypertension and susceptibility to oncogenic disease. For example, our studies in the past have led to techniques which have permitted a far more detailed understanding of the inherited anemia, thalassemia. Current studies involving gene cloning are obviously relevant to the possible production of valuable pharmacologic reagents as well as the diagnosis of certain inherited diseases such as thalassemia and sickle cell anemia.

Proposed Course of Research:

Efforts over the next year will be directed toward cloning and determining the physical map of human heavy and light chain genes. We will also try to understand, from structural analyses, the molecular basis of the heavy chain class switch. Using the human genes as probes, we also plan to investigate the organization of immunoglobulin genes in established and leukemia cell lines arrested at various stages of development. We also plan to extend our analysis of the mouse globin genes to the entire map of both alpha and beta loci, to analyze the structure of several pseudogenes we have already uncovered and to locate and sequence the embryonic globin genes. In addition we plan to use segments of the globin genes to further pinpoint gene sequences that signal transcription promoters, polyA addition and splicing functions.

Publications:

1. Nishioka, Y. and Leder, P.: The complete sequence of a chromosomal mouse alpha globin gene reveals elements conserved throughout vertebrate evolution. Cell 18, 875-882, 1979.

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6. Nishioka, Y. and Leder, P.: Organization and complete sequence of identical embryonic and plascytoma kappa V-region genes. J. Biol. Chem. 255, 3691-3694, 1980.

7. Nishioka, Y., Leder, A. and Leder, P.: An unusual alpha globin-like gene that has cleanly lost both globin intervening sequences. Proc. Natl. Acad. Sci. U.S.A. 77, 2806-2809, 1980.

8. Perry, R.P., Kelley, D.E., Coleclough, C., Seidman, J.G., Leder, P., Tonegawa, S., Matthyssens, G. and Weigert, M.: Transcription of mouse kappa chain genes: implications for allelic exclusion. Proc. Natl. Acad. Sci. U.S.A. 77, 1937-1941, 1980.

9. Leder, P., Konkel, D.A., Nishioka, Y., Leder, A., Hamer, D.H. and Kaehler, M.: The organization and evolution of cloned globin genes. Recent Progress in Hormone Research 36, 241-260, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 01000-01 LMG

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Molecular Genetics of the Major Histocompatibility Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.G. Seidman	Senior Staff Fellow	LMG NICHD
	B. Velan	Visiting Fellow	LMG NICHD
	J.R. Parnes	Guest Worker	LMG NICHD

COOPERATING UNITS (if any)
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SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are attempting to define the genetic basis of cell-cell recognition. As a first step, we plan to characterize the genes encoding the major histocompatability complex--the transplantation antigens--by the use of recombinant DNA technology. Our initial efforts have been directed towards obtaining cloned cDNAs corresponding to these genes. To find these cDNA clones techniques have been developed which allow the rapid screening of hundreds of cDNA clones. We are currently characterizing clones containing beta-2 microglobulin cDNA and are trying to find clones containing H2 cDNA sequences.

Project Description:

Objectives: Our objective is to understand the genetic basis for several cellular recognition events. Initially we will be concerned with the genes encoding the protein antigens that are responsible for transplant rejection. Although the function of these proteins has not been determined, a great deal has been learned about their structure. These proteins are very polymorphic, suggesting that their genes can alter rapidly during the course of evolution. We hope to understand the organization of these genes and their special features that allow for these extensive polymorphisms. Furthermore, we expect to be able to better define the family of related proteins that are responsible for this type of cell-cell recognition.

Methods Employed:

We are using recombinant DNA techniques to study the arrangement of genes encoding several cell surface proteins. The initial step in these procedures has required the generation of hybridization probes that will allow us to identify the genes of interest. These hybridization probes are made by cloning the mRNA sequences for these cell surface proteins. Unfortunately, the individual mRNA species are rare (1:1000-1:10,000). We have developed methods which will allow us to rapidly screen several thousand cDNA clones for the one clone containing the sequence of a particular protein. Using these procedures we expect to be able to clone the mRNA for any protein for which a good antisera is available.

Major Findings:

1. I have finished some experiments that I began while I was still a member of Dr. Leder's laboratory. Normally, kappa light chain genes are formed by a somatic recombination event that joins a V region to a J region. We have shown that when this recombination event takes place, some germline DNA sequences are lost by deletion. Furthermore, the products of an unusual recombination event in a particular myeloma were characterized. A defective gene resulted from this unusual recombination event. Finally, we showed that even though a constant region gene may not undergo recombination it is still transcribed into mRNA.

2. In collaboration with Dr. E. Appella we examined the synthesis of an unusual immunoglobulin heavy chain (alpha). The B47A IgA is missing the fourth domain, however, the mRNA from which this protein is synthesized is not deleted. The defect is probably a point mutation that affects mRNA maturation.

3. To isolate the cDNA clones of interest we have devised a screening procedure which allows us to find a cDNA clone for any mRNA whose protein product can be identified by an antiserum. Using this procedure

we have attempted to isolate a cDNA clone corresponding to beta-2-microglobulin mRNA. Preliminary evidence suggests that we have cloned this mRNA.

Significance to Biomedical Research and the Program of the Institute:
These studies are directed towards understanding fundamental processes in the development of living organisms at a molecular level. More immediately we hope to achieve a better understanding of the phenomena of transplantation rejection. The transplantation rejection phenomena has plagued various attempts to cure human disorders. Organ transplants and marrow transplants are all made much more difficult by the problems of matching donors and recipients. By understanding the genetic basis for transplantation rejections we may hope eventually to achieve better matching systems and screening procedures. Also, as a result of DNA cloning experiments it may become possible to produce pharmacologic agents that will be useful in further studies of the transplantation antigens.

A second benefit of these experiments may derive from the known association of some alleles of the major histocompatibility antigens with different human diseases. For example, over 90% of patients with ankylosing spondylitis have the HLA-B27 allele. Perhaps, with a better understanding of histocompatibility genetics, we may begin to understand the basis of these associations.

Proposed Course of Research:

Efforts over the next year will be directed towards understanding the organization of the mouse and human beta-2 microglobulin and H-2 genes. We plan to clone and characterize the H-2 D and K genes. As a first step we will screen a variety of H-2 antisera for one which will precipitate the H-2 antigens made in an *in vitro* translation reaction. Using this sera we hope to identify an H-2 D or H-2 K cDNA clone. With these cDNA clones we can hope to isolate the appropriate genes from several strains of mice, allowing us to compare the genes and begin to understand the basis of their polymorphisms. Similar studies with the beta-2 microglobulin clone should allow us to analyze this gene(s) in detail. We also hope to examine several cell lines that fail to produce beta-2 microglobulin in the hopes of identifying portions of this gene that are required for its expression.

Publications:

1. Seidman, J.G., Max, E.E. and Leder, P.: A kappa immunoglobulin gene is formed by site-specific recombination without further somatic mutation. Nature 280, 370-375, 1979.

2. Swan, D., D'Eustachio, P., Leinwand, L., Seidman, J., Keithley, D. and Ruddle, F.H.: Chromosomal assignment of the mouse kappa light chain genes. Proc. Natl. Acad. Sci. U.S.A. 76, 2735-2739, 1979.

3. Obata, M., Yamawaki-Kataoka, Y., Takahashi, N., Kataoka, T., Shimizu, A., Mano, Y., Seidman, J.G., Peterlin, B.M., Leder, P. and Honjo, T.: Immunoglobulin lambda-1 heavy chain gene: structural gene sequences cloned in a bacterial plasmid. Gene 9, 87-97, 1980.

4. Perry, R.P., Kelley, D.E., Coleclough, C., Seidman, J.G., Leder, P., Tonegawa, S., Matthyssens, G. and Weigert, M.: Transcription of mouse kappa chain genes: implications for allelic exclusion. Proc. Natl. Acad. Sci. U.S.A. 77, 1937-1941, 1980.

5. Robinson, E.A., Ferrini, V., Seidman, J.G., and Appella, E.: Messenger RNA coding for the deleted heavy chain of mouse myeloma MOPC47A immunoglobulin. J. Biol. Chem. 255, 4988-4991, 1980.

6. Seidman, J.G. and Leder, P.: A mutant immunoglobulin light chain is formed by aberrant DNA and RNA splicing events. Nature, in press.

7. Seidman, J.G., Nau, M.M., Norman, B., Kwan, S.-P., Scharff, M., and Leder, P.: Immunoglobulin V/J recombination is asscompanied by deletion of J and V region segments. Proc. Natl. Acad. Sci. U.S.A., in press.

8. Seidman, J.G., Max, E.E., Norman, B., Nau, M. and Leder, P.: The sources of kappa light chain diversity: A review. Mid-West Autumn Immunology Symposium, Elsevier-North Holland Press, in press.

NICHD Annual Report
October 1, 1979 through September 30, 1980
Child and Family Research Branch

Summary of Research Activities

The past year has been a period of major involvement in data collection. Current studies are investigating 1) the ways in which infants vary in cognitive, motivational and personal-social development, 2) the origins of these variations, 3) the interrelationships among these functions. Some of these issues are being studied in the contexts of cesarean delivery and working mothers, and against the background of the changing role of fathers in early child care. As part of this research, we have developed methods for studying husband's and wife's experience of the childbirth process, methods for obtaining parents' perceptions of infant temperament, procedures for assessing mastery motivation in infants and young children, and methods for studying the complex, reciprocal interactions among mother, father and infant.

In the past year, data collection has been completed on several projects: an investigation of the psychological concomitants of childbirth and their effects on the neonate and on parents' early interactions with the infant, a comparison of the neonatal status of infants born in the hospital with infants born at home; research on the early development of the infant's motivation to explore and to have an impact on the environment, a comparison of mastery motivation in normal infants and infants with Down's syndrome, and a cross-cultural investigation of mother-infant interactions using films. In several other studies, data collection is in progress: a study of the relation between the early mother-infant attachment and later socio-emotional development; research on the continuity of mastery motivation between infancy and the preschool years and the role of the early environment in its development, research on the integration of child care and work roles and its impact on the family; study of the parent-infant interaction subsequent to cesarean delivery.

Much of our data on the psychological concomitants of childbearing have been obtained retrospectively by the woman's report. In a study of child-childbearing, data were obtained through direct observation. Methods were developed to obtain and record the parents' experience of the birth process, other methods were devised to obtain their perceptions of the infant. Some interesting differences emerged from comparisons between the parents' perception of infant temperament with an observer's more objective appraisal. Parents see their infants as more capable of modulating state, and as more vulnerable to stress, observers see infants as more socially alert and responsive to attempts to soothe. Early analyses of the woman's capacity to cope with the stress of childbirth show no direct relationships to the woman's specific expectations of childbirth. These analyses indicate, however, that the ways in which the woman handles stressful events of childbirth are significantly related to her previous history of coping. In another study of the childbirth environment, some significant differences were found

between infants born at home- and hospital-born infants. Home-born infants were were motorically more mature, showed significantly lower levels of tonicity and were more responsive to inanimate stimuli.

Research continues on the development of mastery motivation in early childhood. Data collection has been completed in a study of 6 and 12 month infants, and data analyses are underway. One goal of this research is to develop and refine methods of studying mastery and measures to assess its early manifestations. We are also investigating the impact of the early environment and the mediating role of early temperament in the development of mastery behaviors. We hypothesized that the infant's motivation to explore the environment, to practice emerging skills, to obtain feedback from the inanimate environment and to persist in working on challenging problems are basic for the development of competence, that is, that mastery motivation underlies competent behavior. To test this hypothesis, we analyzed relationships between mastery behavior at 6 and 12 months and performance on the Bayley Scales of Infant Development. Some significant relationships were found between the Bayley indices of competence at 6 months and performance on the mastery tasks. However, separate analyses by sex indicated that the relationships held only for boys, the relationships for girls were negligible. There were, however, no significant differences between boys and girls in level of performance on either the mastery tasks or the Bayley. These findings may indicate different styles of information processing and different patterns of mental development for boys and girls. Further analyses will focus on the relationship between early parent-child interactions and the development of mastery motivation and competence.

A parallel study is being carried on with children with Down's syndrome. Investigation of mastery behavior in children with Down's syndrome revealed basic similarities in some dimensions of mastery. The infants with Down's syndrome showed similar patterns of persistence in working on the tasks. Although the overall level of success was lower for the Down's syndrome babies, they tended to be successful on similar types of tasks as the non-delayed infants. There were, however, no significant relationships between level of exploratory behavior in Down's syndrome infants and competence on the Bayley Scales. These findings suggest that process-oriented assessment of how delayed children approach problems may add to the usefulness of simple scores on cognitive tests. Contrary to other studies which have reported no developmental delays before 6 months in children with Down's syndrome, we found significant delay in performance on the Mental Development Scale as early as 3 months. This developmental retardation becomes more pronounced with increasing age. Further analyses are underway on the mother's perception of the infant's temperament and on mother-infant interaction.

In progress is a short-term longitudinal study designed to investigate the continuity of mastery behavior during the first 2 1/2 years of life and to study the relationships between parent's behavior at 6 and 12 months and later mastery in their children. Techniques have been developed for studying mastery in preschool children; these techniques attempt to assess behaviors at 2 1/2 years that are conceptually similar to those measured

in infancy. Data are also being obtained on the infant's cognitive development to ascertain whether mastery motivation in infancy is related to the development of cognitive competence in the preschool years.

Another short-term longitudinal study is concerned with the relationship between infant-mother attachment and later socioemotional development. Children who were first studied at 6 and 12 months in the mastery study are being assessed between their fifteenth and eighteenth months: 1) the validity of the construct of attachment is being investigated in two ways. determining whether early measures of the quality of attachment are predictive of later socioemotional development, and 2) comparing two measures of the construct, security of attachment. A situational test, developed in this branch, the Differential Social Reaction Procedure, is being compared with a measure, the Strange Situation, developed by Ainsworth. A significant relationship between these two independent measures of quality of attachment was found, thus indicating some cross-situational validity for the construct. Infants classified as insecurely attached in the Strange Situation showed less preferential response to the mother in the Differential Social Reaction Procedure than infants classified as securely attached. Data on the predictive value of these two measures for later social and emotional adaptation are being obtained.

Cesarean childbirth is a potentially stressful surgical procedure that may have important psychological consequences for both infant and parents. Although cesarean deliveries have shown a marked increase in recent years, there has been relatively little research on the psychological impact of this experience on the family. In progress is a study aimed at describing the range of immediate adaptive behaviors which families show subsequent to cesarean childbirth, and to determine the longer term impact on parental behavior and the infant. Fifty families are being studied at 3 and 6 months, 20 of these families have experienced a cesarean delivery. The impact of this event on the husband-wife relationship and the father-infant interaction is being studied. Data are being obtained through direct observation of the families as well as in interviews with the parents. In addition, the parents' perception of the baby's temperament is being obtained by a procedure developed for a previous study in the branch.

A wide variety of opinions exist about the impact on the family and on the child of the mother's working during the early period of the child's life. Despite this controversy, an increasingly large number of mothers are entering the labor force. Underway is a study of how families with young infants reach decisions about maternal employment, and how the mother's employment status influences the interactions of both parents with an infant. Data are being obtained on the factors influencing the mother's decisions to seek employment, also whether systematic differences exist between mothers who are employed by the time the infant is 3 months of age, mothers who return to work when the baby is between 3 and 12 months, and mothers who are employed during the first year of their baby's life. Of particular interest is the impact of these variations in the mother's employment status on the father-infant relationship. Some hypotheses suggested by a pilot study will be investigated. Although it was anticipated

that in families in which both parents worked, fathers would show higher rates rates of interaction with the infant, this was not found to be the case. In families where mothers worked, fathers played less with their infants, and mothers engaged in more focused social play and greater verbal interaction.

A cross-cultural pilot study has been completed based on comparison of film records of the Fais in Micronesia with similar film records of caregivers and children in America. The purpose of this study was to examine the usefulness of films as sources of data for studying caregiver-infant interactions, and to develop procedures for continuous coding of these filmed interactions. The findings indicate that film records can yield rich data for cross-cultural studies. It was found, for example, that infants in Fais interact with a greater variety of caregivers and in more varied contexts than in the United States. The kinds of interactions that occur are in turn significantly influenced by both context and caregiver. Infants and caregivers were more likely to be in the presence of others and had less face-to-face contact with caregivers in Fais than in the United States. These findings suggest that crosscultural differences in infant's experiences are attributable in part at least to differences in the social contexts in which infants and their caregivers are most often found in a given culture.

Professional Activities of Staff:

Members of the staff have been involved in many professional activities. They have been members of boards of national foundations such as the Easter Seal Research Foundation, the National Center for Clinical Infant Programs, the professional advisory council of Parent and Child Association. They have served on the editorial boards of a number of journals: The Merrill-Palmer Quarterly Journal of Behavior and Development, the Monographs of the Society for Research in Child Development, Family Relations. In addition, they have served as ad hoc reviewers for Child Development, Developmental Psychology, the Psychological Bulletin, Infant Behavior and Development, the International Journal of Behavioral Development, the Journal of Applied Psychology, and the Journal of Child Psychology and Psychiatry. They have served as ad hoc reviewers of research grant proposals for the Canada Council, National March of Dimes, the National Science Foundation,, the National Institute of Mental Health, the National Institute for Neurological Diseases and Stroke, the Nursing Division of NIH, the National Institute on Drug Abuse. Staff members have been participants in a conference on behavioral research in chronic disease sponsored by the National Cystic Fibrosis Foundation. They have given presentations to the Department of Psychiatry, University of California at Los Angeles, and have participated in a symposium at the Educational Testing Service. They have served as consultants to the Pediatrics Department, Vanderbilt University and the University of Cincinnati, the American Society for Psychoprophylaxis in Obstetrics.

Publications Not Directly Related to any Specific Projects:

Altemeier, W. A., Vietze, P. M., Sherrod, K. B., Sandler, H. M., Tucker, D. D., and O'Connor, S. Prediction of child maltreatment in pregnancy. In S. Harel (Ed.), Selected papers from the international workshop on the "At Risk" Infant. Excerpta Medica: Amsterdam, 1980, pp. 225-228.

McLaughlin, F. J., Vietze, P. M., Sherrod, K., and O'Connor, S. Social-psychological characteristics of adolescent mothers and behavioral characteristics of their first-born infants. Journal of Population, 1979, 2(1), 69-73.

Messer, D. J. The episodic structure of maternal speech to young children. Journal of Child Language, 1980, 7, 29-40.

Publications Not Directly Related to Specific Projects (Cont'd.):

Suwalsky, J. T. D., Martin, P. P., Fivel, M., and Jennings, K. D. Code for assessing level of dyadic peer interaction. JSAS Catalog of Selected Documents in Psychology, 1980, 10, (MS #2061), 37.

Vietze, P. M., Altemeier, W. A., Falsey, S., Sandler, H. M., and O'Connor, S. Prospective study of mothers at risk for child maltreatment. In S. Harel (Ed.), Selected papers from the international workshop on the "At Risk" infant. Excerpta Medica: Amsterdam, 1980, pp. 62-66.

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Watson, J. S., Hayes, L. A., and Vietze, P. Bidimensional sorting in preschoolers with an instrumental learning task. Child Development, 1979, 50, 1178-1183.

Watson, J. S., Hayes, L. A., Lorman, L., and Vietze, P. Infant sex differences in operant fixation with visual and auditory visual reinforcement. Infant Behavior and Development, 1980, 3, 107-114.

Yarrow, L. J. Development of the child's personality in the first five years. In J. G. Howells (Ed.), Modern perspectives in the psychiatry of infancy. New York: Brunner/Mazel, 1980, pp. 1-24.

Yarrow, L. J. Emotional Development. American Psychologist, 1979, 34(10), 951-957.

Yarrow, L. J. Interazione fra i rapporti che coinvolgono il bambino in via de sviluppo. In Cesa-Bianchi (a cura), Aspetti biosociali dello sviluppo, Vol. 1. Milano: Franco Angeli, 1979, pp. 177-182.

Zaslow, M. J. Relationships among peers in Kibbutz toddler groups. Child Psychiatry and Human Development, 1980, 10(3), 178-189.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00012-04 CFR

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Development of Mastery Motivation in Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Vietze	Senior Staff Fellow	CFR, NICHD
	L. J. Yarrow	Chief, Child & Family Research	CFR, NICHD
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LAB/BRANCH

Child and Family Research Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.25	4.00	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of the project is to investigate the development of mastery motivation in the first year of life. In a previous study, (Z01 HD 00008-04) three aspects of mastery were distinguished: persistence in problem solving, in practicing emerging sensorimotor skills, and in obtaining feedback from the physical environment. The interrelationships among these components of mastery motivation are being explored. The origins of mastery in parent-child transactions and its continuity over time are being studied. The sample includes 75 infants from middle income families. Several methods are being employed--a series of twelve tasks designed to measure mastery motivation, observation of mother-infant and father-infant interaction at home, Bayley Scales of Infant Development and several self-report measures of parent-infant interaction. Analyses will focus on continuity in mastery behavior between 6 and 12 months and on the relationship between the development of mastery and parent-infant interaction.

Project Description:

Objectives: The major goal of this project is to investigate the ontogeny of three facets of mastery motivation during the first year of life: persistence in problem solving, in practicing emerging sensorimotor skills, and in obtaining feedback from the physical environment. This research builds on a previous investigation in which measures of mastery motivation were developed for 12-month-old infants (Z01 HD 00008-04). In the present study we have revised and extended these measures, and have developed methods for studying mastery at a younger age, at 6 months. A second purpose of the project is to examine the environmental correlates of mastery motivation. We have observed mother-infant and father-infant interaction when the infants are 6 and 12 months of age to ascertain the significance of the infant's early experiences with parents and with objects for the development of mastery motivation.

Methods Employed: The subjects include 75 first-born infants from intact middle-income families (39 boys and 36 girls), only infants born after uneventful pregnancies and deliveries were included. Infants and parents were studied when the infants were 6 months of age and again when they were 12 months old. Data were obtained in several sessions at each age. During the first session, the infants were given the Bayley Scales of Infant Development; during the second laboratory visit, six of the mastery motivation tasks were administered; the other six tasks were given on the third visit. To allow for estimates of the internal consistency of the procedure, the order of presentation of the two sets of tasks was counter-balanced. Within a week of the laboratory sessions three observations of parent-infant interaction in the home were made. In addition, the parents were asked to keep a diary of the infant's activities for a week and to complete an inventory designed to obtain their perception of the infant's temperament.

The three components of mastery motivation were assessed in a variety of ways. The Practicing Sensorimotor Skills component consists of tasks in which it is necessary to coordinate perceptual and fine motor skills. An example of one of the items for 6 month olds is a toy in which three plastic "men" in a small tub are presented, the infant's task being to remove the men from the tub and then replace them. A similar task for 12 month olds is a peg boat from which the infant can remove pegs and replace them. The Problem Solving tasks include a variety of situations in which an object is located out of direct reach of the infant, but within view. The infant is encouraged to obtain the object. For the 6 month olds, one of the problem solving tasks consists in presenting an attractive toy just beyond the infant's reach. The toy is on a piece of cloth and can be obtained only by pulling the cloth towards him. Effectance Production tasks are those in which the infant produces an effect by his own action. At 6 months, one of the Effectance Production tasks consists of an Activity Center in which a variety of effects are produced by turning knobs, spinning wheels or pushing levers. Each task is presented for three minutes and is videotaped for later scoring. Both frequency and duration of behaviors, such as

attempting to complete the task, strategies used to explore the objects, amount of exploratory behavior and the type of affect accompanying the tasks are coded.

Observations of parent-infant interaction: An observational system which attempts to preserve the sequence of interaction between the parent and infant has been developed. The observer focused on the dyad with reference to communicative behaviors, caretaking, and the object-related behaviors of each member. These observations were made without the observer having any knowledge of the infant's performance on the Mastery Assessment or the Bayley Scales. To establish observer reliability observations were made by two observers simultaneously at regular intervals during the study. The observers used an electronic digital recording device, the Datamyte, consisting of a small 14 button keyboard through which the coded data are entered and stored in the unit along with the time of each data entry.

Major Findings: Data collection for the longitudinal study has been completed. Data analyses for all components of the project are underway. Analyses completed thus far reveal a number of interesting findings. These findings may be considered preliminary in that further analyses may lead to refinement of these results. Findings will be reported for several aspects of the home observations of parent-infant interaction and for the relationship between performance on the Mastery Motivation Assessment and Bayley Development Scales.

With Regard to Parent-Infant Interaction,

The data derived from the 450 observation sessions of mother-infant and father-infant interaction were reduced to proportions of the session that each behavior was observed to occur. In general, it was found that at both 6 and 12 months of age, there was great similarity between the parents. This was also true of infant's behavior in the presence of each parent. However, a number of interesting findings emerged when some of the individual behaviors were combined to form more meaningful variables. The variables were analyzed using repeated measures analyses of variance. The mothers were found to spend more time in caregiving, although the differences at 12 months were not great. Both parents spent more time in caregiving activities at 6 months than at one year. When caregiving was analyzed into different activities some interesting differences were found. Fathers spent twice as much time as mothers soothing their infants although neither parent spent much time in this activity. Mothers, on the other hand, spent more time in activities such as feeding, changing and bathing. These differences were not very large, perhaps a reflection of a more equitable division of the work of child care in these families than has been found in previous research.

Fathers spent more time than mothers gazing at their infants and more time actively stimulating and interacting with objects when they were with the babies. Analysis of the facial/communication variables yielded some unexpected findings. The fathers also tended to spend more time than the mother near the baby and holding the infant. Subsequent analyses will focus on how these proximity differences affect other interactional measures.

In general, the data show high involvement of the fathers with their first born infants in both practical and social aspects of parenting. The results support other findings that fathers are involved in playful interactions with their babies.

As part of the information on the families some data were available on the conditions of birth. In keeping with the national trend, we found a relatively high proportion, 23%, of the infants in our sample, had Cesarean deliveries. In order to examine the effects of Cesarean childbirth on mother- and father-infant interaction, we examined the variables outlined above for the Cesarean and vaginally delivered infants. We found the only effect of Cesarean delivery on the infant's behavior was that at six months they fussed more with their fathers than with their mothers. At 12 months there were no differences in infant behavior. Thus, in the present middle income sample, there are few consequences of mode of delivery evident by 6 months.

Effects of mode of delivery on parental behavior were found. Both parents showed more caregiving, and feeding at 6 months and more stimulation behavior at both ages if the infants were born by Cesarean section. These differences are especially interesting inasmuch as there were no differences between the two groups in the infant behaviors monitored. It is possible that parents felt that their cesarean-delivered infants needed more caregiving, another possibility is that any early infant behavioral differences related to mode of delivery led to more caregiving by the parents and this parental behavior persisted in spite of changes in the infant behaviors. The findings for the fathers provide some replication of the findings of another study being carried on in the Branch (Z01 HD 00015-03). Cesarean delivery seems to increase the fathers involvement in the care of their infants. The more extensive soothing shown by fathers of the Cesarean infants at 6 months may be a result of the increased fussiness shown by these infants. However, the fathers' increased participation in caregiving may be a response to the fact that the mothers who deliver by Cesarean take longer to recover than those who deliver vaginally.

In regard to the effects of mode of delivery on mothers' behavior in interaction with first-born infants, were few behaviors other than caregiving distinguished mothers of cesarean and vaginally delivered infants at 6 months. However, at 12 months the mothers of the cesarean infants had increased their attention, and showed more holding and touching of these infants. It is possible that these mothers feel more distant from their infants at first which may lead to less holding, touching and attending. Pedersen's and Zaslow's study (Z01 HD 00015-03) may provide further data on these issues.

Mastery Motivation Assessment

At present the 12 month mastery motivation assessments are being coded. The coding will be completed by the end of FY 1980. The 36 categories used in coding have been collapsed to form six categories of behavior: visual attention, three levels of exploratory behavior, social orientation, and

non-task behaviors. In examining the data for the infants at 6 months across all the tasks, it was found that infants spent 92% of their time engaged in task relevant behavior. They were engaged in one of the three levels of exploratory behavior. The first level consisted of simple exploration of the task materials, such as touching, mouthing, dropping, holding and examining the materials. The second level included activities which involved more complex exploration, such as relating parts of the materials to one another in more adaptive ways. The third level of exploration included only behaviors which had the potential of solving a problem, producing an effect or successfully completing the activity. These third level behaviors are considered Mastery behaviors. When the 6 month infants' bebehavior on these three levels of exploratory behavior were examined for all tasks combined it was found that they engaged more in the two lower levels of exploratory behavior, 28% and 32% of the time respectively, and engaged in mastery behaviors only 21% of the time. These results indicated that, the 6 month old infants are most likely to master tasks in which the major feature is the production of effects and least likely to master problem solving tasks in which they must obtain a hidden object. Mastery in the fine motor skills tasks fell in a position between the other two types of tasks. It will be interesting to see how these results compare to results for the twelve month mastery assessment.

As one way to validate the mastery assessment, the relationships between performance on the mastery tasks and performance on an independent test of infant development were examined. Correlations were computed between the variables described above and the two scaled scores derived from the Bayley Scales of Infant Development, the Mental Development Index (MDI) and the Psychomotor Development Index (PDI). Since these latter measures were available for the infants at both 6 and 12 months, the correlations were calculated for both ages on these two scores. Initial results yielded several significant but moderate correlations between mastery behavior (level 3) and the two Bayley scores at 6 months and also for level 1 exploration and the two 6 month measures. The only significant correlation between 12 month MDI and the mastery assessment was with level 1 exploratory behavior. At 6 months the correlation between mastery and the Mental Development Index was as predicted. When the same measures for each of the three types of tasks were correlated with the Bayley scores, it was found that mastery behavior on the Effectance Production tasks and the Fine Motor Skills tasks was related to mental development at both 6 and 12 months and also to 6 month motor development. There were also significant correlations for level 1 exploratory behavior and mastery behavior on the Fine Motor Skills tasks with the Bayley scores. None of the relationships between Problem Solving and Bayley scores were significant. Analysis of the 12 month Mastery data is expected to clarify further interrelationships between Mastery behavior and cognitive development.

Separate analyses for boys and girls indicated few significant correlations between mastery and performance on the Bayley for the girls. For the boys, however, there were significant correlations between these two sets of measures.

Multivariate analyses have been carried out to assess the relationship between the combined frequency and duration scores of the mastery tasks and the Bayley test scores. These multiple correlations show stronger relationships between the mastery assessment and the results of the Bayley testing than either duration or frequency measures alone.

Significance to Biomedical Research and the Program of the Institute: Although the major analyses dealing with the relatedness between parent-infant interaction, mastery motivation and infant competence have yet to be analyzed, a number of analyses of other facets of the research have yielded significant findings. The findings that fathers have a major role, comparable to that of mothers, in providing caregiving for their infants in the first year document cultural changes in roles of parents. The analyses of effects of mode of delivery on parent-infant interaction indicate how Cesarean delivery impacts on postnatal events.

The discovery that exploratory behavior yields stronger relationships to cognitive competence for boys than for girls suggests that there may be different styles of information processing for boys and girls. These findings may have implications for understanding subsequent gender differences in socialization especially as they relate to school achievement.

Proposed Course of Project: The unscored video records will be coded by October 1980. Completion of data analysis should follow and the results of initial analyses will be presented in a number of reports. A more extensive analysis of the interaction data is presently being planned.

Papers Presented at Professional Meetings:

Vietze, P. M., MacTurk, R. H., McCarthy, M. E., Klein, R. P., and Yarrow, L. J. Impact of mode of delivery on father- and mother-infant interaction at six and twelve months. Presented at the Second International Conference on Infant Studies, New Haven, Ct., April, 1980.

Vietze, P. M., and Marcus, B. C. Longitudinal changes in mother-infant interaction in the first year. Presented at the Sixth Biennial Southeastern Conference on Human Development, Alexandria, Va., April, 1980.

Publications:

Vietze, P. M., and Hopkins, J. B. Mother-infant interaction: A model for intervention. In R. R. Abidin (Ed.), Parent Education Handbook. Springfield, Ill: Charles Thomas, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00013-04 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Role of Mother-Infant Relationship in Subsequent Socio-emotional Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. Klein	Research Psychologist	CFR, NICHD
Other:	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	M. E. McCarthy	Research Assistant	U. of Maryland
	N. F. Gist	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)
None

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	2.0	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to identify factors in infancy which are predictive of later socio-emotional development. This project will focus primarily on the aspects of the mother-infant relationship and infant functioning which are hypothesized to be important for healthy emotional development during early childhood. In the second study of this project we have obtained several measures of the mother-infant relationship at the beginning of the infant's second year. We are currently following these infants up through their fifth birthday. Analyses of the data already collected have provided additional evidence for the validity of the Differential Social Reaction Procedure which was developed in this Branch.

Project Description:

Objectives: A major goal of this longitudinal study is to test the validity of the construct of attachment. Two major questions are being asked. Do measures of attachment in infancy predict later socioemotional development? Are independent measures of the quality of attachment significantly related?

Methods Employed: In order to replicate the findings of the first study in this project a second study has begun using subjects who participated in another study of mastery motivation (Z01 HD 00012) when they were 6 and 12 months of age. The children are seen twice with their mothers between their 15th and 18th months. The first session employs the Differential Social Reaction Procedure which was developed as a part of a previous project (Z01 HD 00005). In addition the infants' social overtures to an unfamiliar adult and their mothers are observed during free play. The second session consists of the Strange Situation, a standardized procedure developed by Mary Ainsworth for measuring the quality of the infant-parent relationship. The Strange Situation consists of eight episodes of increasing stress, including two separations from the parent. Of particular interest are the infant's behaviors when the parent returns. On the basis of these and other behaviors infants are classified into one of three groups: insecurely attached/avoidant, securely attached, and insecurely attached/resistant. These children will be followed up until their fifth birthday. Every six months during this time we will obtain information from the parents regarding their child's development and stressful events which have occurred to their child. In addition we will obtain reports from nursery school teachers for those children who become enrolled in a preschool. This follow-up information will allow us to examine the extent to which socio-emotional development is dependent on the quality of the mother-infant relationship and the extent to which it is dependent on contemporaneous stresses the child experiences.

Major Findings: Of the 75 children enrolled in the previous study we have collected mother-infant relationship data on 48. Although we obviously are not yet able to carry out analyses addressing the main purpose of the study (the extent to which measures of the mother-infant relationship at the beginning of the second year of life predict social-emotional development through age five), we did have sufficient data to examine the intercorrelations among our various measures obtained between 15 and 18 months. We were particularly interested in comparing the scores from the Differential Social Reaction Procedure with security of attachment as assessed by the Strange Situation because Harmon, et al. reported evidence that the Differential Social Reaction Procedure also yields information regarding security of attachment. The results from the second study have confirmed these findings from the first study. Specifically, infants classified as insecurely attached by the Strange Situation showed less preferential behavior to the mother than to the stranger. They were less likely to avoid the stranger and more likely to avoid their mother in the Differential Social Reaction Procedure than were infants classified as

securely attached. No differences were found between the securely and insecurely attached infants on their approach to the stranger in the Differential Social Reaction Procedure. These findings lend further support to our assumption that presence or absence of preference for mother in this situation is indicative of the expectations infants have formed about their mothers.

Significance to Biomedical Research and the Program of the Institute: On the basis of experience with emotionally disturbed individuals clinicians from several different theoretical perspectives have affirmed that the early mother-infant relationship is crucial for later emotional and cognitive development. There has, however, been little consensus on how to assess this relationship meaningfully. This project has provided one method of assessment along with evidence for its validity. Future data collection in this project will subject this method and the other major method, the Strange Situation, to further validity testing by examining their correlates with later socioemotional development.

Proposed Course of Project: A third study has been started which will assess the infants' relationships with their fathers as well as with their mothers. In this study extensive information on the infants' experience with substitute caregiving will be obtained, thus permitting study of the influence of this important dimension of experience on the parent-infant relationship. We will also continue to collect follow-up information on infants who have already been seen in the parent-infant relationship phase of data collection.

Publications:

Klein, R. P., Durfee, J. T., and Fivel, M. W. Possible methodological influences on intercorrelations among attachment behaviors. Merrill-Palmer Quarterly, 1979, 25, 293-298.

Harmon, R. J., Suwalsky, J. T. D., and Klein, R. P. Infants' preferential response for mother versus an unfamiliar adult: Relationship to attachment. Journal of the American Academy of Child Psychiatry, 1979, 18, 437-449.

Klein, R. P., and Yarrow, L. J. Maternal behavior and sharing by toddlers. Psychological Reports, 1980, 46, 1057-1058.

Klein, R. P., and Jennings, K. D. Responses to social and inanimate stimuli in early infancy. Journal of Genetic Psychology, 1979, 135, 3-9.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00014-04 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Psychological Aspects of Childbearing and the Neonatal Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Standley	Research Psychologist	CFR, NICHD
OTHER:	J. Nicholson	Research Assistant	U. of Maryland
	N. F. Gist	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)
Department of Obstetrics and Gynecology, The Washington Hospital Center, Washington, D.C.

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: 2.25	PROFESSIONAL: 2.00	OTHER: .25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Childbirth is a special interaction of physiology and psychology which may have important consequences in infant development. The objective of this research is to gain understanding of psychological aspects of the childbirth experience and their impact on the well-being of the child, the new parents, and the family unit.

The current goals of this research are to develop instruments which allow for objective and comprehensive measurement of childbirth events. A method has been developed for recording the physical state of the woman in labor and medical and social interactions with her. A measure of delivery room events is now being developed. Prenatal and postpartum interviews with parents provide data on their expectations and recollections of the birth process which can be compared with the researcher's observations. Parent's perceptions of the newborn are compared with the researcher's more objective assessment of infant behaviors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Childbirth represents an interface of physiology and psychology which may have important consequences for the physical and emotional health, not only of the infant, but of the parents as well. The objective of this research is to develop methods and gather data which contribute to an understanding of the psychological factors in childbirth. These measures are both subjective and objective, from participants as well as researchers, and hopefully contribute a more comprehensive view of the psychology of childbearing than has previously been available.

Methods Employed: The subjects are forty middle-income, Caucasian couples and their first-born infants. Data were obtained at three points in the perinatal period. In the last month of pregnancy, the expectant mother was interviewed about her pregnancy experience and her expectations of birth and parenting. Demographic and historic data were also obtained.

During each woman's labor, a member of the research staff observed the woman and her husband for one hour and recorded labor room events in accordance with a time-sampling method developed for this purpose. The observation system taps various dimensions of the woman's physical state and all medical and social events which impinge on her. The observer watches the woman and others for 30 seconds and then records the observed data for 30 seconds. The system has provided a precise and comprehensive record of the labor room experience. Information was also acquired from hospital records.

One week after the birth, another member of the research staff visited the new parents in their home and interviewed them about their recollections of the birth and their new parenting experiences. These data are being compared with the researcher's actual observations of the labor. Parents also completed the Newborn Behavior Inventory which obtains their perceptions of the baby's temperamental characteristics. The NBI was developed in the course of this project. The dimensions of the NBI correspond to the Neonatal Behavioral Assessment Scale which was given independently by a trained examiner. As with the measures of the childbirth setting, two views of the infant can be compared--the parent's and the researcher's.

Major Findings: Several measures developed in the course of the research have been published or presented at meetings. These methods include a system for observing and recording during labor, a companion system for observation of delivery room events; the Newborn Behavior Inventory for measuring parental perceptions of infants; and prenatal and postpartum interview schedules for assessing parental expectations and recollections of childbirth.

These data indicate that mothers, fathers and examiners differ in their perceptions of the behavioral characteristics of newborns. Compared with examiners, parents tend to see their babies as more capable of modulating state and controlling motor behaviors, they also see their infants as more physiologically vulnerable to stress. On the other hand, examiners

perceive the infant as more capable in social interaction in an alert state and more responsive to consoling. Examiners appeared to draw on the salient aspects of their interactions with the neonate while parents seemed to focus on the physical well-being of their infant and minimize the interactive potential of the newborn.

The analyses still to be carried out will provide a more comprehensive picture of the childbirth experience. A preliminary between-measure analysis taps key variables of the prenatal and postpartum interviews and the ratings of the woman's behavior during labor and the use of pain-relieving medications during labor and delivery. Demographic factors such as woman's age predict observed coping behaviors during childbirth while factors reflecting the woman's history of coping with stress, especially during pregnancy, are more predictive of indices of physical coping and postpartum memories of coping with the childbirth. Specific expectations of childbirth are not significantly related to subsequent coping.

In an analysis of a subset of the data, we looked at ways childbirth events influence women's beliefs about locus of health control. Beliefs about patterns of reinforcement with respect to health--whether dictated by the woman herself (Internal), by powerful others (Others), or perceived as relatively unordered (Chance)--were measured using Form A of the Multidimensional Health Locus of Control Scale (MHLC). This scale was given to the women before and after childbirth.

Prenatally, high scores for the women on the Internal Scales were significantly related to positive reports of supportive woman-physician relationships, relationships with friends, enthusiasm for the pregnancy experience and a feeling of enhanced physical well-being during pregnancy. Subscale change scores were calculated by subtracting postpartum scores from corresponding prenatal scores. Changes on the Internal Subscales were positively associated with increased incidence of medical intervention and the administration of analgesia. Change on the Chance Subscale was related to interpersonal happenings rather than medical events. The findings indicate that childbirth events influence women's beliefs about their own roles and the role of chance in influencing health.

Significance to Biomedical Research and the Program of the Institute: Childbirth is increasingly recognized as a critical event in the early psychological development of the child and the new family. It is hoped that the data of this research program and continuing research in behavioral perinatology will contribute significantly to pediatric and obstetric health care and to parents' understanding of and involvement in their childbearing experiences.

Proposed Course of Study: Data analysis will be completed and the findings will be published.

Publications:

Standley, K. Research on childbirth: Toward an understanding of coping. In P. Ahmed (Ed.), Coping with pregnancy, in press.

Standley, K., and Nicholson, J. Observing the childbirth environment: A research model. Birth and the Family Journal, 1980, 7(1), 15-20.

Standley, K., and Gist, N. Maternal stress and pregnancy outcome: Commentary. ICEA Review, 1980, 4(1), 8.

Van der Waals, F., and Standley, K. Manual for naturalistic observation of the childbirth environment: Part II. JSAS Catalog of Selected Documents in Psychology, 1980, 10:37 (MS 2035).

Papers Presented at Professional Meetings:

Nicholson, J., and Standley, K. The impact of electronic fetal monitoring on the psychological experience of childbirth. Presented at the 107th Annual Meeting of the American Public Health Association, New York, New York, November, 1979.

Van der Waals, F., and Standley, K. Obstetrical care in the United States and the Netherlands: A contrast in health care delivery. Presented at the 107th Annual Meeting of the American Public Health Association, New York, New York, November, 1979.

Nicholson, J., Gist, N, van der Waals, F., and Standley, K. Research on Childbirth: Understanding psychosocial and physiological factors influencing perinatal health. Presented at the Association for Advancement of Behavior Therapy and Society of Behavioral Medicine Annual Meetings, San Francisco, December, 1979.

Nicholson, J. Naturalistic observation of the childbirth environment: New research. Presented at the National Convention of the American Society for Psychoprophylaxis in Obstetrics, Waterville, New Hampshire, July, 1980.

Nicholson, J. Social and emotional issues of pregnant adolescents: How to adapt your current program to meet their needs. Presented at the National Convention of the American Society for Psychoprophylaxis in Obstetrics, Waterville, New Hampshire, July, 1980.

Nicholson, J. Childbirth events and changes in maternal health locus of control. To be presented in Symposium on Issues of Control and Health Annual Meetings of the American Psychological Association, Montreal, Canada, September, 1980.

Nicholson, J. Participant in a panel discussion on "Birth by Lamaze: Where have all the psychologists gone?" To be presented at the annual meetings of the American Psychological Association, Montreal, Canada, September, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00015-03 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Parent-Infant Interaction Subsequent to Cesarean Delivery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Pedersen	Research Psychologist	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	R. L. Cain, Jr.	Research Psychologist	CFR, NICHD
	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	B. A. Rabinovich	Research Assistant	U. of Maryland
	E. Kramer	Student Scientist	CFR, NICHD

COOPERATING UNITS (if any)
Parent and Child, Childbirth Education Associates, University of Maryland

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to examine the effects of cesarean childbirth on family interaction and on the parent-infant relationship. The sample consists of 50 families with a medically normal, first-born infant, 20 having had a cesarean delivery and 30 a vaginal delivery. When the babies are three months old, and again when they are a year old, two observations are made of mother, father and baby interacting, and one observation is made of mother and baby alone. At each age parents are interviewed, asked to complete a Q-sort assessing their perceptions of their baby's temperament, and to record their participation in household and childcare tasks on a weekday and a day on the weekend. Cross-sectional and longitudinal analyses will assess differences in parents' reports of their experiences during childbirth, in family interaction, and in their adjustment to parenthood subsequent to different birth experiences.

Project Description:

Objectives: Although the rate of cesarean delivery among middle-class mothers has more than doubled in the past decade, there have been relatively few focused investigations of the psychological impact of this experience on family relationships. The purpose of the present study is to obtain longitudinal data on the mother-infant, father-infant and spouse relationships in families where there was a cesarean delivery. Three major research questions are addressed: (1) what is the range of variation in adaptation to cesarean childbirth shown by parents of medically normal infants? (2) Compared to families that had a vaginal delivery, is cesarean birth associated with different behavioral patterns in relationships among mother, father, and infant? (3) If there are areas of difference, what is the longitudinal course of these patterns during the first year of life?

Methods Employed: The present investigation seeks to avoid some of the methodological deficiencies in the few previous investigations on this topic. The study will be unique in several respects: (1) It addresses cesarean childbirth specifically rather than as an accident of a research design directed to other problems, thus yielding more focused information. (2) It will study a broad range of effects, including relationships that might be influenced indirectly by the birth experience, e.g., father-infant or husband-wife interaction. (3) Methods include self-report and observational techniques, allowing comparisons of effects at a behavioral level as well as in the cognitive constructions of the experience. (4) The investigation is longitudinal, allowing some appraisal of the time boundaries of effects. (5) The infants will be screened to eliminate cases with postnatal complications, which might confound the effects of different birth experience.

The sample consists of 20 families with a medically normal, cesarean delivered infant and a comparison group of 30 families with a normal vaginally delivered infant (drawn from another study - Z01 HD 00016 CFR). The groups will be comparable on all pertinent background variables, e.g., parental age and education, years of marriage, maternal employment status, and the sex of the infant. All infants will be first-born.

Observational procedures from a previous study (Z01 HD 00003 CFR) of mother-infant, father-infant, and mother-father interaction have been revised for use in the present study. In addition an extensive parental interview has been developed which addresses the following areas: (1) the birth experience and adjustment to parenthood; (2) division of household and child care tasks and (3) employment history, attitudes and plans about employment. A previously developed method for studying parents' perceptions of their babies' temperament has been revised and extended for the present study. A time log has been developed to record each parent's participation in household and child care tasks on a weekday and a day on the weekend. Finally, an interview has been developed to determine the frequency of mother-infant separations and arrangements for substitute care during the infant's first year.

Families are seen when the infant is 3 months old and again when the baby is 12 months old. At each age there are two observations of mother, father, and baby together, and one observation of mother and baby. At each age parents are interviewed, asked to complete the perception of infant temperament measure and the time log. When the infants are 6, 9, and 12 months old the interview concerning mother-infant separations is carried out by telephone.

Major Findings: A pilot study, based on six cesarean delivered infants and a comparison group of 17 infants drawn from another project (Z01 HD 00003 CFR) yielded a number of findings which suggest hypotheses for this investigation. In spite of the very small sample, significant differences were found at age 5 months between parents with different childbirth experience. Father-infant caregiving and father responsiveness to the infant's distress appeared to be enhanced subsequent to cesarean childbirth. Purely social interaction between father and infant seemed diminished in comparison to vaginally delivered infants. Relatively few differences related to birth experience were evident in mother-infant interaction except that mothers and cesarean delivered infants displayed less reciprocal positive affect in their interaction and these mothers engaged in robust physical handling of their babies less frequently than mothers of vaginally delivered infants. The main conclusion of the pilot study was that both father and mother appeared influenced by the birth circumstances. Although formal data analysis for the present investigation has not begun, our impression is that several hypotheses generated by the pilot study will be verified.

Significance to Biomedical Research and the Program of the Institute: A cesarean childbirth is a potentially stressful surgical procedure that may have important psychological sequelae. It is important to determine whether distinctive patterns of care and interactions with the baby emerge as a consequence of this experience. The present study will clarify the range of adaptations which occur following cesarean childbirth, and determine whether differences persist throughout the infant's first year. The study, by identifying areas of maternal and paternal behavior that are affected, will have important implications for intervention and prevention programs.

Proposed Course of Project: Enrollment of participants in the study has been completed and all of the 3 month data collection will be completed by the end of this fiscal year. During the next fiscal year, analyses of the 3-month data will be underway and the 12-month assessments will be completed. Longitudinal analyses will then be initiated. It is anticipated that preliminary reports of findings will be available in the next fiscal year.

Papers Presented at Professional Meeting:

Pedersen, F. A., Zaslow, M. J., Cain, R. L., & Anderson B. J. Cesarean childbirth: The importance of a family perspective. Paper presented at a symposium entitled "The Psychological Impact of Cesarean Childbirth" at the International Conference on Infant Studies, New Haven, April, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00016-05 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Integration of Child Care and Work Roles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Pedersen	Research Psychologist	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	R. L. Cain, Jr.	Research Psychologist	CFR, NICHD
	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	B. A. Rabinovich	Research Assistant	U. of Maryland
	E. Kramer	Student Scientist	CFR, NICHD

COOPERATING UNITS (if any)
Parent and Child, Childbirth Education Associates, University of Maryland

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.75	3.50	.25

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to obtain longitudinal descriptive data on various adaptation patterns which families use to integrate child care and employment in the infant's first year of life. Three groups of families will be studied: those in which the mother plans to be the full time caregiver for the infant during its first year; families in which the mother plans to be employed before the baby's first birthday, but is not employed when the baby is 3 months old, and families in which the mother is employed by the infant's 3 month birthday. At 3 months and again at 12 months, two observations are carried out in the families' homes when mother, father and baby are together and one observation is carried out when mother and baby are alone. Parents are also interviewed, asked to complete a Q-sort to measure their perceptions of their infant's temperament, and asked to complete a time log of a weekday's and weekend-day's participation in household and child care tasks. Data analysis will consist of cross-sectional and longitudinal comparisons of the three groups.

Project Description:

Objectives: Mothers with very young children are being employed in unprecedented numbers. This study attempts to address a number of questions about mothers' employment decisions in families with first born infants:

1. What factors are related to mothers' decisions to seek employment or to be full-time caregivers in the infant's first year of life?

2. Are there systematic differences in the behavior of mothers with different employment patterns, i.e., mothers who are already employed when the infant is 3 months old, mothers who expect to be employed by their baby's first birthday, and mothers who plan to provide full time child care over the same period of time?

3. Is the father-infant relationship affected by variation in the mother's employment role?

4. What is the longitudinal course of each parent's relationship to the baby when there are different maternal employment patterns?

Methods Employed: The sample will consist of approximately 50 families with first born infants, divided among three groups: mothers who are employed when the baby is 3 months old; mothers who plan to be employed before the baby's first birthday, but are not yet employed when the baby is 3 months old; and mothers who plan to be the baby's caregiver throughout its first year. Families will be seen when the baby is 3 months old and again when the baby is 12 months old.

Observational procedures from a previous study (Z01 HD 00003-05 CFR) which were designed to obtain data on mother-infant, father-infant, and mother-father interaction, have been revised for the present study. A parent interview has been developed which addresses several topics: the experience of becoming a parent, the division of child care and household tasks between mother and father; employment history, plans and attitudes of the mother and father. A previously developed method for studying parents' perceptions of their baby's temperment has been revised and extended for the present study. A time log has been developed for each parent to record participation in household and child care tasks on a weekday and a day on the weekend. Finally, an interview has been developed to determine the extent of mother-infant separations and arrangements for substitute care over the infant's first year.

Major Findings: A pilot study, based on observations of 5-month-old infants from another project (Z01 HD 00003 CFR), has yielded a number of hypotheses to be studied in the present investigation. We found that both parents' behavior with the infant are influenced by the family's role organization. Compared to families in which the mother is a full-time caregiver, mothers who work outside the home showed much greater verbal interaction with their babies. Moreover, they also engaged in focused social play more frequently than mothers who are full-time caregivers.

Fathers whose wives were employed showed a significant diminution in rates of play with their babies, compared to fathers whose wives are full-time caregivers. Although we expected to see more egalitarian patterns of child care and social behavior in two-wage-earner families, this was not the case. The present investigation will provide important information on the longitudinal course of these behavior patterns.

Although formal data analyses are just beginning, two impressions stand out: (1) The clear secular trend toward maternal employment during the infancy period has heightened the emotional intensity surrounding the choices which parents are making. Some of these issues were developed in a paper titled "Childbearing and Sex Role Choice," by Martha Zaslow and Frank Pedersen. (2) Maternal employment patterns are highly differentiated, including variation in whether employment involves separations from the baby, the nature of the work being pursued, its financial rewards, and the patterns for maintaining job involvement and controlling the timing of job reentry. Although our research design focuses on three major groups, we have identified many more differentiated patterns of "maternal employment."

Significance fo Biomedical Research and the Program of the Institute: This study will provide insight into how families with young infants reach decisions about maternal employment, and how maternal employment influences interactions of both parents with an infant. These findings will help assess the impact on families of mothers' employment decisions.

Proposed Course of Project: Enrollment of participants in the investigation has been completed and all of the 3-month-data collection will be completed by the end of this fiscal year. During the next fiscal year, analyses of the 3-month-data will be underway and the 12 month assessments will be completed. Longitudinal analyses will then be initiated. It is anticipated preliminary reports of finding will be available in the next fiscal year.

Papers Presented at Professional Meetings:
Pedersen, F., Cain, R., Zaslow, M., & Anderson, B. Variation in infant experience associated with alternative family role organization. Paper presented at the International Conference on Infant Studies, New Haven, April, 1980.

Publications:
Pedersen, F. A. (Ed.) The father-infant relationship: Observational studies in the family setting. New York: Praeger, 1980.

Zaslow, M., & Pedersen, F. Childbearing and sex role choice. American Psychologist, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00017-03 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Cross-Cultural Comparison of Caregiver-Infant Interactions Using Film Records: Pilot Phase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Vietze	Senior Staff Fellow	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	A. Sostek	Asst. Prof. of Psychology in Pediatrics	Georgetown University
	L. Kreiss	Anthropologist	NINCDS
	F. van der Waals	Visiting Fellow	CFR, NICHD

COOPERATING UNITS (if any)
National Institute of Neurological and Communicative Disorders and Stroke, NIH and Georgetown University

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .25	PROFESSIONAL: .25	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This project examines the usefulness of cross-cultural films from existing archives for studying the nature of caregiver-infant interaction in different cultures. Sixteen sequences of caregiver-infant interaction from films of Fais, an island in Micronesia, have been identified for analysis. For cross-cultural comparison, 13 sequences of American infants and caregivers were filmed in naturalistic contexts. A system for continuous coding of non-verbal social behaviors of caregivers and infants has been developed, and both sets of film have been coded using this system. Cross-cultural comparisons revealed that infants in Fais were less likely to be in face-to-face contact with caregivers than infants in the United States sample. Further, social context differed between the cultures. In Fais, caregivers and infants were more likely to be in the presence of others. Analyses revealed that in each culture, many behaviors were affected by the number of people present with the caregiver and infant. These findings suggest the possibility that cross-cultural differences in infants' experiences are attributable at least in part to differences in the social contexts in which infants and their caregivers are most often found in a culture.

Project Description:

Objectives: The major objectives of this study are:

1. To develop a procedure for continuous coding of caregiver-infant behaviors that can be used with film or videotapes in cross-cultural studies;
2. To examine the usefulness of films made in a number of cultures as sources of data for the study of caregiver-infant interaction,
3. To examine whether interactions with infants have such strong demand-characterisitics that cross-cultural differences in non-verbal interactions are minimized.

Methods Employed: Based on an observational system previously used with digital recording devices, a method has been developed to describe nonverbal social behaviors of infants and caregivers using film records. Sixteen segments, each of at least one minute in length, have been selected from a file record taken by a visual anthropologist (D. Rubinstein) on the island of Fais in Micronesia. Films of infants under 2 1/2 years of age in proximity to a caregiver were studied. The recorded behaviors included categories of infant visual and manual activity, posture, facial expression, and the varieties of stimulation provided by the caregiver. In addition, a number of interactive behaviors were recorded, e.g., mutual visual regard and play.

To provide comparison data, 13 sequences of American infants and their caregiver have been filmed and coded using the same procedure. Both the Fais and the American films include sequences in which infant and caregiver are alone, and sequences in which they are in the presence of others.

Major Findings: Two sets of analyses have been carried out: cross-cultural comparisons of caregiver-infant interaction, and within culture analyses of variation in behavior according to number of people present.

Cross-cultural analayses. Because mean infant age differed for the Fais and the United States sample, cross-cultural comparisons were made with infant age as a covariate. Analysis of covariance was applied to each variable as a proportion of its overall category (e.g., "manipulates object" as a proportion of all infant manual activity). Despite the large number of variables examined, cross-cultural differences were found only for gaze and head orientation of the infants. Infant and caregiver faced one another more frequently in the United States. In Fais, the most common head orientation was for the infant to be facing out in the same visual arc as the caregiver. American caregivers looked at the infants significantly more. In Fais, the most common pattern pattern was for caregiver or infant not to be looking at the other.

Context analyses. Within each culture, there were substantial differences in interaction according to number of people present. For analyses of context, the film segments were divided into three groups: those in which the caregiver-infant dyad was alone, those in which between one and three other people were in proximity to the dyad (small group) and those in which

more than three other people were present (large group). In America, infrequent large groups made it necessary to restrict comparisons to dyads alone versus small groups. Age was again treated as a covariate.

In both cultures, the small group context was characterized by less intense interaction between caregiver and infant. Although adults in this context used more expressive facial expressions (e.g., playface and grimace), close holding was infrequent and the infant's eyes were more likely to be directed at a part of his or her own body. When dyads were alone, two patterns emerged. Either intense face-to-face interaction occurred (U.S. and Fais), or the infants were more likely to wander away from the caregiver (U.S. only). In the large group (which occurred only in Fais) infants tended to be held, and face-to-face positioning was significantly diminished.

Significance to Biomedical Research and the Program of the Institute: Much of the empirical support of theories of early social development has been obtained in Western cultures. This pilot investigation was designed to examine the feasibility of using ethnographic film libraries as sources for observational studies of non-Western cultures. This would be especially important in studying cultures which have undergone transitions but for which film records exist. Our findings suggest that this is a viable technique for studying non-Western or primitive cultures, when there are extensive supporting field notes to document information about the subjects filmed, and when ethnographers' filming strategies are known.

The results of this study point to the importance of social context as a determinant of infants' interaction with a caregiver. The caregiver-infant dyad is not the prevalent social setting on Fais or in many other non-Western cultures. Our findings indicate that interaction with a caregiver tends to differ according to number of people present. Thus, a major factor to consider in understanding cross-cultural differences in infants' experiences is which social context or contexts are prevalent in a culture. Research that controls for context (e.g., observing only caregivers and infants in dyadic interaction) may overlook important cross-cultural variations.

Proposed Course of Study: Findings have been presented at two scientific meetings. The results will appear in a book on culture and early interaction. This work also provoked an examination of methodological issues in cross-cultural studies of interaction, and a chapter on this issue will also appear in a book on culture and early interaction. An invited article on the feasibility of using cross-cultural film archives as sources of data on caregiver-infant interaction is in process.

Papers Presented at Professional Meetings:

Sostek, A. M., Vietze, P., Zaslow, M., Kreiss, L., van der Waals, F., and Rubinstein, D. Social context in caregiver-infant interaction: A film study of Fais and the United States. Paper presented at the Sixth Biennial Southeastern Conference on Human Development, Alexandria, Va., April 17-19, 1980.

Papers Presented at Professional Meetings (Cont'd.)

Zaslow, M., Sostek, A. M., Vietze, P., Kreiss, L., van der Waals, F., and Rubinstein, D. Cross-cultural differences in expressiveness in caregiver-infant interaction: An analysis of filmed behavior on the Micronesian island of Fais and in the United States. Presented at the meetings of the Society for Cross-cultural Research, Charlottesville, Va., February, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00019-02 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mastery Motivation and Social Competence in Down's Syndrome Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Vietze	Senior Staff Fellow	CFR, NICHD
OTHER:	M. E. McCarthy	Research Assistant	U. of Maryland
	S. McQuiston	Research Assistant	U. of Maryland
	R. H. MacTurk	Research Assistant	U. of Maryland

COOPERATING UNITS (if any)

Institute for Child Study, University of Maryland, Children's Hospital, Children's Brain Research Clinic

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	0.75	0.10

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this research is to study social competence and mastery motivation in the first year of life of infants with Down's syndrome and to examine the relationship between the mother-infant interaction and infant's mastery behaviors. Down's syndrome infants are being studied longitudinally at 3, 6 and 8 months, and a cross-sectional sample is being studied at 12 months of age. Mastery motivation is being assessed using tasks which have been developed in this laboratory. An observational system which preserves the sequence and continuity of the transactions between mother and infant is being employed to examine the mother-infant interaction. In addition, the relationship between the infants' social and mastery behaviors and specific cognitive, and temperamental variables is being examined. Early findings highlight similar performance on cognitive-motivational variables between Down's infants and normal infants. Differences between these two groups in face-to-face interaction have been found.

Project Description:

Objectives: The major purpose of this investigation is to study the interractions with the social and physical environment of infants with Down's syndrome in the first year of life. Specifically, Down's syndrome infants' communicative competence in social interaction and mastery motivation in interaction with inanimate objects is being examined. Using measures of mastery and social interactive behaviors which have been developed in this branch, the project is designed to examine the development of these behaviors in Down's syndrome infants and to specify the manner in which their development differs from that of normal infants. In addition, we are interested in examining the relationship between specific cognitive, and temperamental variables and the Down's syndrome infant's mastery and social interactive behaviors. We expect that the information gained from the study of social and cognitive-motivational development in Down's syndrome infants will be useful for the development of intervention programs.

Methods Employed: Infants with Down's syndrome and their parents are being recruited through two physicians in Washington, D.C. and through parent-infant education programs in the Washington, D.C. metropolitan area. Both the longitudinal and cross-sectional samples will consist of 30 infants. Data on a comparable number of normal infants are also being obtained. Participants in the longitudinal phase of the study are seen in our laboratory at 3, 6, and 8 months of age. At 3 months, observations of mother-infant interaction are made, the Bayley Scales of Infant Development are administered, and the mother's perception of her infant's temperament is obtained through a Q-sort technique. At 6 months, mother-infant interaction is observed, and the infants are administered the mastery motivation tasks and the Bayley Scales of Infant Development. At 8 months, the mastery tasks and Bayley Scales are administered. Infants in the cross-sectional portion of the study visit the laboratory on three occasions at 12 months of age and are administered the mastery motivation tasks and the Bayley Scales. The infant's performance on the mastery tasks is video-taped for later coding using observational procedures which have been developed for another study (Z01 HD 00012-04).

The mother-infant interaction observations consist of a laboratory procedure in which the infant and mother are video-taped while facing one another with the infant seated in an infant seat before the mother. These video-tapes have been coded using a preliminary version of the final set of categories which will be utilized when all the data have been collected.

Major Findings: The total sample of infants with Down's syndrome has been recruited. For comparison with this group, demographically matched nondelayed infants are being studied. Thirty infants and their mothers have been seen in our laboratory on at least one occasion. Preliminary analyses have been carried out with regard to developmental level mastery behavior, mother-infant interaction and maternal perception of temperament.

Developmental Testing

Infants with Down's syndrome have been characterized as exhibiting no developmental delays through the first 6 months with a rapid deceleration in progress thereafter. Analysis of the available data on the Bayley Scales of Infant Development indicates that as early as 3 months, the infants exhibited significant delay in performance on the Mental Development Scale, which becomes more pronounced with age. For the Motor Development Scale, there were no significant delays at three months. However, between 8 and 12 months of age the infants showed significant increase in delayed motor functioning. In general, performance on both mental and motor scales showed a steadily increasing divergence from theoretical age-appropriate development during the first year. Use of a standardized test to measure the level of developmental functioning of infants is a common practice. Although the Bayley Scale of Infant Development is the most carefully developed and standardized test, it shares a problem of most standardized instruments which are normreferenced tests. There are a restricted number of items at any particular age level which might account for the developmental delays as early as we have found them. The fact that most instruments of this sort are product-oriented rather than process-oriented may limit their usefulness for populations of retarded children.

Mastery Motivation

Videotapes of the mastery motivation assessments have been coded for all the 6 and 8 month old infants tested to date. The coded results have been reduced from 36 categories of behavior to six categories, reflecting three levels of exploratory behavior, as well as visual attention to materials, social orientation and non-task relevant behavior. The data have been summarized for all tasks. Preliminary analyses showed slight differences between the 6 and 8 month infants in visual attention to objects and in task related behavior in general. In both cases, the older infants spent less time looking at and more time interacting with the objects. When the three levels of exploratory behavior were considered, the patterns of results were quite similar for the Down's syndrome and non-delayed infants, that is, the distribution of time the infants spent on the tasks across the three levels of exploratory behavior was similar.

Mother-infant Interaction

Four infant behaviors and two maternal behaviors were coded. For the Down's group, the infants showed an increase in the incidence of head orientation and gaze toward the mother and of smiling, between 3 and 6 months. Infant vocalization did not change significantly across this period. The mothers, on the other hand, showed no changes in behavior between the time their infants were 3 and 6 months of age. In addition, at 6 months the infants smiled less often during the imitation sequence than during the play sequence. Comparisons of the Down's and normal infants and mothers also revealed a number of interesting differences. The normal infants smiled and vocalized considerably more often than did the Down's infants and the mothers of the Down's infants were significantly more active than the non-delayed infants' mothers.

These preliminary findings confirm previous reports that mothers of Down's syndrome infants are more active when playing with their infants than mothers of nondelayed infants. This difference can be observed as early as 3 months. Asking mothers to imitate their infants seems to reduce their activity level and hence allows the infant to have a chance to take a more active role in the interaction. During the imitation phase of the session there is increased infant vocalization. It has been shown elsewhere that young infants, whether delayed or not, tend to cease vocalizing when someone else is talking. It would seem that the imitation sequence leads the mothers to interact more responsively with their Down's infants than they do when unrestricted by such instruction.

Maternal Perception of Temperament

Infants with Down's syndrome have often been described as having easy-going, placid temperaments. They have also been stereotyped as being affectionate, friendly and lovable. Studies of institutionalized children with Down's syndrom substantiate these characterizations. However, there has been little effort to examine systematically the mothers' perceptions of their Down's syndrome infants temperaments. Our preliminary analyses compared the mother's view of the infant's temperament in the normal and Down's samples. These comparisons were made when the infants were 3 and 6 months of age. At each age differences were found on only two of the eleven scales. The mothers perceived their 3 month old Down's infants as lower in approach to new situations than did the mothers of non-delayed infants. They also saw the Down's infants as being less responsive to environmental events and more adaptive than the mothers of the nondelayed infants. These results for Approach and Adaptability, support the traditionally reported stereotype of the Down's child. However, the fact that there were no differences on the Activity, and Intensity scales does not support the view that Down's infants are flaccid and floppy. The lack of difference on the Positive Mood and Easy/Difficult scales also is counter to the stereotype.

There are two alternative explanations for these results. It is possible that the traditional stereotypes apply to institutionalized older children with Down's syndrome but not to home-reared infants. It is also possible that the mothers are not accurate reporters of their infants' temperaments. Comparisons of the mothers' reports with objective judgments made by indepedent observers will reveal the areas in which there are discrepancies.

Significance to Biomedical Research and the Program of the Institute: This project has already uncovered some important aspects of Down's syndrome infants' behavior heretofore not reported. Systematic analysis of exploratory behavior may provide key data which will allow parents and professionals who have contact with Down's syndrome infants to interact with them more effectively. The preliminary analyses of mother-infant interaction suggest that it might be useful to study an intervention program in which parents imitate their infants. Some studies indicate that increased vocalization is associated with later language development.

Proposed Course of the Project: Data collection will be completed by the fall, 1980 and the analysis of data among the different domains will continue for 8 months following this time. Results already presented are being prepared for publication.

Papers Presented at Professional Meetings:

MacTurk, R. H., Vietze, P. M., McCarthy, M. E., and McQuiston, S. Emergence and trends of performance decrements among Down's Syndrome infants. Paper presented at the annual meeting of the American Association on Mental Deficiency, San Francisco, Ca., May, 1980.

McCarthy, M. E., MacTurk, R. H., Vietze, P. M., and McQuiston, S. Maternal perception of infant temperament in families of Down's Syndrome infants. Paper presented at the annual meeting of the American Association on Mental Deficiency, San Francisco, Ca., May, 1980.

McQuiston, S., McCarthy, M. E., MacTurk, R. H., and Vietze, P. M. Mother-infant interaction with Down's Syndrome infants. Paper presented at the annual meeting of the American Association on Mental Deficiency, San Francisco, Ca., May, 1980.

Vietze, P. M., McCarthy, M. E., MacTurk, R. H., McQuiston, S., and Yarrow, L. J. Exploratory behavior among infants with Down's Syndrome. Paper presented at the annual meeting of the American Association on Mental Deficiency, San Francisco, Ca., May, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00020-02 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Impact of Childbirth Setting on Infant Behaviors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Standley	Research Psychologist	CFR, NICHD
	N. F. Gist	Research Psychologist	CFR, NICHD
OTHER:	J. Nicholson	Research Assistant	U. of Maryland

COOPERATING UNITS (if any)
Dept. of Obstetrics & Gynecology, Washington Hospital Center, Wash., D.C.
Maternity Center Associates, Bethesda, Maryland

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research project is investigating the importance of the childbirth environment for newborn functioning. The Brazelton Neonatal Behavioral Assessment Scale was administered to healthy, one-week-old infants born in planned home deliveries. These infants were compared with a group of hospital born infants drawn from a current study of hospital births ("Psychological Aspects of Childbearing and the Neonatal Period," Z01 HD 00014-04). An effort will be made to determine whether there are differences in the two birth environments, and to identify aspects of these environments, such as specific obstetric practices, which may account for differences in home and hospital born infants. Of special interest are the effects of obstetric medications on the behavioral and neurological functioning of the newborn.

Project Description:

Objectives: Because of the increasing numbers of expectant parents who are electing to have planned home deliveries, this study was designed to study the relative advantages and disadvantages of home births and hospital births. The behavioral characteristics of infants born at home will be compared with a comparable group of infants born in traditional hospital deliveries.

Methods Employed: The methods of this study are taken from the larger research "Psychological Aspects of Childbearing." In addition, the comparative data of the hospital born sample are taken from that study.

Two measures of the behavioral characteristics of the newborn infant are employed in this study. When the infant was 7 to 10 days of age the mother and father were given the Newborn Behavior Inventory, an instrument that obtains the parents perceptions of the child, and the Brazelton Neonatal Behavior Assessment Scale which was administered by a trained examiner.

In order to control for differences that might be due to the use of analgesic drugs in the hospital, comparisons were made between the home born infant and two hospital samples--one sample of infants born in entirely unmedicated births and one sample whose mothers were given epidural anesthesia.

Major Findings: When home born infants were compared with infants who were born in the hospital to non-medicated mothers, some significant differences emerged. Home born neonates were more responsive to inanimate stimuli. They showed significantly lower levels of tonicity and were motorically more mature.

Comparisons of the two hospital groups, one born to mothers who received no obstetric medication and the other group born to mothers who received epidural anesthetic, revealed that the newborns whose mothers were unmedicated were significantly more responsive to animate visual stimulation (the examiner's face), and were more alert.

These analyses are continuing in more detail, comparing parental reports of the newborn using the Newborn Behavior Inventory with the examiner reports from the Brazelton Neonatal Behavior Assessment Scale.

Significance to Biomedical Research and the Program of the Institute: The home birth movement of health consumers is not only making home births a viable alternative in several parts of the country, but it is creating social and economic pressures for change in more traditional obstetrics. This study addresses the issue of whether the birth setting makes a difference to the newborn.

Proposed Course of Study: Data Collection of the hospital born sample and the home born sample has been completed and analyses are continuing.

Paper Presented at Professional Meetings:

Gist, N. F., and Standley, K. A comparison of behavior in hospital and home born neonates. Paper presented at the American Public Health Association's 107th Annual Meeting, New York, New York, November 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 01100-01 CFR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
A Follow-up Study of Mastery Motivation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. J. Yarrow	Chief, CFR Branch	CFR, NICHD
	D. J. Messer	Visiting Fellow	CFR, NICHD
OTHER:	M. E. McCarthy	Research Assistant	U. of Maryland
	B. C. Marcus	Research Psychologist	CFR, NICHD
	R. H. MacTurk	Research Assistant	U. of Maryland
	D. Rachford	Research Assistant	U. of Maryland

COOPERATING UNITS (if any)
Institute for Child Study, University of Maryland

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.4	0.10

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study of the longitudinal course of the development of mastery motivation, the goals are to determine whether mastery motivation in infancy is predictive of later mastery behavior and whether specific aspects of parent-infant interaction influence mastery behavior at 2 1/2 years. The interrelationships between the development of cognitive functions and mastery motivation during the preschool years is also being studied. Methods have been developed to assess similar aspects of mastery at 2 1/2 years as were studied at 6 and 12 months of age. Data are being collected using these measures of mastery, the McCarthy Scales, an observation of the infant's free play with the mastery toys, and a brief observation of mother-child interaction with another set of toys.

Project Description:

Objectives: This study is concerned with the longitudinal course of cognitive development and mastery behavior during the first 2 1/2 years of life. The overall goals are to determine whether mastery motivation assessed in infancy is predictive of later mastery behavior and whether parent-child interaction in infancy is predictive of later manifestations of mastery behavior. The children in this sample were first studied at 6 and 12 months of age (Z01 HD 00012-03) and are now being assessed at 2 1/2 years.

Method Employed: The subjects are 69 first-born 2 1/2 year old infants, 37 boys and 32 girls. Subject loss has been very low, with 94% of the mothers who have been contacted agreeing to participate. Methods have been developed to assess similar aspects of mastery at 2 1/2 years as were studied at 6 and 12 months of age. Data are collected in two sessions. During the first session, the children are given a standardized intelligence test, the McCarthy Scales of Human Abilities. Afterwards they take part in a task involving the operation of some simple levers which switch on a contingent display. During the first half of the second session six toys are presented by the examiner to the child one at a time, giving the child an opportunity to explore and attempt to master the toy. Next, four of the toys are reintroduced and the child is allowed to play with them as he or she wishes. In the second half of the session, mother and child are observed playing together with another set of toys.

Major Findings: During this year, pilot work was carried out in a day care center and later in our testing rooms. The pilot work was for several purposes: to select the tasks which were appropriate for this age group, to standardize the presentation of the McCarthy Scales and to finalize details of the mastery procedures. Logic circuits were also built for the contingency task so that the reinforcement could be presented automatically and the sequence of lever presses could be recorded. Data collection has been completed on 35 subjects.

Codes have been developed for analyzing the childrens' behavior on the mastery tasks. A system has been devised for rating mastery behavior on the McCarthy Scale tasks. A coding system is also being devised for the contingency task to record the child's attention to the task, exploration and help-seeking, all of which relate to mastery motivation. Data analysis for the structured mastery tasks and the free play is also progressing. A preliminary coding system has been developed to record the sequence and duration of play. Progress has been made in finalizing some of the codes and of processing the recordings that have been made. Computer programs have been designed to determine the reliability of the coding systems and to analyze the data.

Significance to Biomedical Research and the Program of the Institute: The child's motivation to explore and master the environment is thought to be both a precursor and an element of cognitive development. If appropriate measures can be developed to assess mastery motivation during infancy and the preschool years, then the longitudinal course of mastery motivation can

be studied. If we can ascertain factors in the infant and in the early interaction with parents that facilitate the development of mastery behavior, it might be possible to influence its course. This project also has implications for theories of continuity of behaviors and for early influences on personal-social and cognitive development.

Proposed Course of Project: Data collection will be ended by March 1981. During this time the coding system will also be completed. Data analysis is likely to continue until the summer of 1981.

1980 ANNUAL REPORT
Pregnancy Research Branch

Project Numbers	Project Title	Principal Investigator
Z01 HD 00026-05 PR	Fertilization and Activation of Development in Mammals	B. Gulyas
Z01 HD 00029-06 PR	In Vitro Studies on Primate Luteal Cells	B. Gulyas
Z01 HD 00031-06 PR	Hormonal Interrelationships Between Fetus, Mother and Placenta in Primates	G. Hodgen
Z01 HD 00045-05 PR	Regulation of Folliculogenesis in the Monkey	G. Hodgen
Z01 HD 00168-04 PR	Ovarian Polycyclic Aromatic Hydrocarbon Metabolism, Reproductive and Oocyte Toxicity	D. Mattison
Z01 HD 00900-02 PR	Factors Regulating Estrogen and Progesterone Receptors in Monkey Endometrium in the Fertile Menstrual Cycle	G. Hodgen
Z01 HD 00901-02 PR	The Core Endocrine Assays Laboratory	W. Nixon
Z01 HD 00906-01 PR	Metabolism of Steroids and Zenobiotic Compounds by Microsomal Monooxygenases	D. Mattison
Z01 HD 00907-01 PR	Reproductive Toxicity of Drugs	D. Mattison
Z01 HD 00908-01 PR	Genetics of Murine Atresia	D. Mattison
Z01 HD 00909-01 PR	Effects of Ethanol on the Developing Mammalian Fetus	A. Mukherjee
Z01 HD 00910-01 PR	Possible Role of Uteroglobin, Transglutaminase and Progesterone in the Success of an Allotransplantation in Nature: Mammalian Pregnancy	A. Mukherjee
Z01 HD 00911-01 PR	Superovulation Induced Intrauterine Growth Retardation in Mice	A. Mukherjee
Z01 HD 00912-01 PR	Gene Regulation and Cellular Differentiation	J. Chou
Z01 HD 00913-01 PR	Primate Models for In Vitro Fertilization and Alternatives	G. Hodgen
Z01 HD 00914-01 PR	Fallopian Tube Dysfunction	D. Hodgen
Z01 HD 00915-01 PR	Allogeneic Bone Transplantation In Utero	M. Michejda

ANNUAL REPORT
of the
Pregnancy Research Branch
National Institute of Child Health and Human Development
October 1, 1979 through September 30, 1980

SUMMARY

The current research program of the Pregnancy Research Branch focuses upon the physiology of gestation (including genetic and developmental factors which contribute to perinatal fetal and maternal morbidity and mortality), fertility regulation, toxicological aspects of ovarian function, as well as fertilization and implantation. Both basic and clinical aspects of the reproductive process are being investigated. Besides these research activities, the Branch trains Associates and Visiting Fellows in laboratory and clinical investigation by participation in the combined program in Reproductive Endocrinology that has been established with the Armed Forces Institute, specifically for career development in obstetrics and gynecology. Also, the combined program with NIAMDD is available to Clinical Associates interested in Endocrinology. In addition, the Pregnancy Research Branch has applied jointly with the Department of Obstetrics and Gynecology, Georgetown University to establish sub-specialty certified Fellowships in Maternal-Fetal Medicine and Gynecologic Endocrinology.

We have continued to study cellular differentiation and gene regulation in placenta and liver, to isolate and characterize selected enzyme systems and other proteins from normal placenta, choriocarcinoma cells, SV40-transformed placental cells and SV40-transformed rat liver cells, and to characterize the hormonal regulation of glycogenolysis and gluconeogenesis in liver. Ectopic production of these fetal proteins may serve as an early signal of malignant transformation and serve to elucidate the control mechanisms for the synthesis of these proteins.

Certain polycyclic aromatic hydrocarbons destroy oocytes in rodent ovaries. The mechanism of oocyte toxicity appears to depend on ovarian metabolism of these hydrocarbons to reactive intermediates which are toxic to oocytes. In recent years these studies have proceeded in a mouse model, but is now being expanded to nonhuman primates. Preliminary results show susceptibility of monkey oocytes to polycyclic aromatic hydrocarbons, re-enforcing the probability of significant human risk from these reproductive toxins. Other studies of reproductive toxins include: 1) the metabolic affects on steroids and xenobiotic compounds by microsomal monooxygenases; and 2) side effects of medicinal agents on reproduction, such as cyclophosphamide.

The state of immune privilege in pregnancy, whereby the non-rejection of the fetus is accomplished, remains an enigma. To ascertain the immunosuppressive role of uteroglobin in the rabbit, its affects on the incorporation of H^3thymidine into maternal lymphocytes in a mixed culture with

mitomycin-C inactivated blastomeres was assessed. The mixture of maternal lymphocytes with rabbit blastomeres suggested maternal recognition of fetal antigens, but which is fully suppressed in the presence of uteroglobin and transglutaminase beforehand. The uteroglobin affect persisted after inhibition of transglutaminase, suggesting a uteroglobin crosslinkage to H-2 antigen (embryonic) which masks maternal recognition.

Fertilization, and the sperm-egg interaction which is permissive to it, were investigated in both rodent and primate models. In rodent studies, the cortical reaction and its dependence on ovoperoxidase to achieve zona hardening was studied. The results indicate the presence of an ovoperoxidase, perhaps of cortical granule origin, on the surface (histochemically detected) of activated mammalian eggs. Perhaps a peroxidase catalyzed cross-linking of the tyrosines in the zona causes hardening of the zona pellucida. In primates studies on in vitro fertilization, we aspirated oocytes from the preovulatory follicle, achieved in vitro fertilization and modest success at embryo transfer. An alternative to in vitro fertilization, low tubal ovum transfer, was also developed, allowing upper tubal obstructions to be by-passed. These approaches may enhance clinical investigations aimed at alleviating infertility due to irreparable tubal dysfunction.

The physiological control of ovarian function and endocrine regulation during the reproductive cycle in pregnancy was extensively analyzed in the monkey, as a model for understanding of ovarian function in the primate. The regulation of ovarian folliculogenesis, and selection of the dominant ovarian follicle, was examined during the primate ovarian cycle. Circulating patterns of gonadotropin and ovarian steroids were measured by radioimmunoassay before and after manipulations that included follicle cautery, luteectomy, and hemiovariectomy during the monkey menstrual cycle. In addition to these studies on follicle growth during the non-fertile menstrual cycle, the resumption of follicle growth was investigated in these animals during the puerperium. The relationship between ovarian function and the conceptus, as well as the cessation of follicle growth during pregnancy, were also studied during the fertile menstrual cycle.

The ovarian follicle destined to ovulate was selected by the mid-follicular phase, at the expense of other follicles in the original cohort. The time-course of new follicle growth after removal of the dominant follicle was unaffected by the concurrent removal of one ovary. The next follicle destined to ovulate was not selected until after the removal of the reigning dominant follicle. In the presence of the dominant follicle, the contralateral ovary contributes little if anything to the regulation of follicle growth, ovulation, corpus luteum function, or gonadotropin secretion.

Recently, it was found that charcoal-treated bovine and porcine follicular fluid (PFF1) reduced peripheral plasma FSH levels, with little alteration in LH levels, in gonadectomized rats and in cycling female rats and

monkeys. The data suggested the presence of a nonsteroidal substance (inhibin) in follicular fluid thought to be responsible for regulation of FSH secretion. Administration of porcine follicular fluid to suppress circulating FSH offers a convenient, novel system for evaluating the actions of FSH during the ovarian cycle. In current studies we are testing the effects of PFF1 administration during the first half of the menstrual cycle on follicle development in the rhesus monkey. The goal of this research is to test the hypothesis that the formation and steroidogenic activity of luteal tissue may be impaired, when the corpus luteum originates from a developing follicle exposed to less-than-normal FSH.

In vitro assessment of rhesus monkey (Macaca mulatta) luteal cell function was further extended utilizing dissociated cells from the corpus luteum. The main topics of interest in this project have included 1) morphological and functional correlations of luteal cells maintained in culture for ten days, 2) Effects of non-gonadotropin hormones and cholesterol on the maintenance of functional luteal cells, 3) evaluation of progesterone production by dissociated luteal cells after brief exposure to trypsin, and 4) localization of LH/hCG receptors of dissociated luteal cells.

Having learned that secretions of the fetal pituitary are essential to normal growth and development of ovaries and testes in utero in rhesus monkeys, we now set out to examine the secretory capacities of the fetal and newborn monkey hypophysis. Specifically, we will begin by characterization of FSH and LH concentrations in fetal and infant serum at various "ages". Tests are being conducted to examine both negative and positive feedback conditions which regulate gonadotropin secretion in utero. Current studies will show the evolution of fetal gonadal receptors for LH/hCG during organogenesis. Also, injections of GnRH agonists and antagonists will be used to examine functional dynamics of the hypothalamic-pituitary-gonadal axis at various gestational ages. Finally, studies to monitor fetal stress acutely after exceeding the placental reserve will lead us toward early diagnosis of intrauterine growth retardation.

The major cause of human infertility in the U.S.A. is tubal dysfunction. Endometriosis and other causes of failure to achieve satisfactory gamete transport are major contributing factors. We sought to examine how these infertilities arise and how to better treat them medicinally and surgically. The cause of infertility when endometrial tissue grows ectopically is unknown; even its hormonal support is poorly understood. We studied: 1) the role of estrogens and progesterone in the maintenance of ectopic endometrial plaques; and 2) the effect of ectopic endometrium on ovum and sperm transport. Current projects are aimed at determining if ovum pick-up at ovulation and tubal transport of both egg and sperm are inhibited by ectopic endometrium.

As illustrated by the studies summarized in this report, the investigators within the Pregnancy Research Branch have developed a broad range

of studies on normal processes and disorders which affect fertility and gestation. A variety of animal models and clinical interests are being pursued with the expectation that useful information will be derived about the basic causes and optimal treatment of disorders adversely affecting the reproductive process, especially pregnancy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00026-05 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Fertilization and Activation of Development in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Bela J. Gulyas	Senior Investigator	PRB, NICHD
Other:	Eli D. Schmell	Staff Fellow	PRB, NICHD
	Lydia C. Yuan	Chemist-Technician	PRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 3/4	1 1/2	1/4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An ovoperoxidase is present in the cortical granules of mouse oocytes. Following cortical reaction ovoperoxidase is detectable either in the perivitelline space or on the surface of the zona pellucida. The enzyme is not detectable in the presence of peroxidase inhibitors. Results indicate that the ovoperoxidase is apparently responsible for the hardening reaction of the zona pellucida following cortical reaction. This conclusion is based on the following observations: (1) both ovoperoxidase activity and zona hardening are blocked by several known peroxidase inhibitors; (2) tyrosine analogs, which should competitively inhibit peroxidase catalyzed cross-linking of protein-bound tyrosines, also prevent hardening; and (3) horseradish peroxidase partially hardens the zona of unactivated eggs.

Project Description Z01 HD 00026-05 PR

Objective

The objectives of this project that was initiated a little more than a year ago were as follows: 1) elucidate on the mechanism(s) involved in the triggering of cortical reaction and its consequence in cell surface changes in mammals; 2) define some of the molecules that are involved in the species specific sperm-egg recognition in mammals.

The present experiments were designed to demonstrate the presence of a mammalian ovoperoxidase in activated mouse eggs, and to elucidate on the role of the secreted peroxidase in physiological changes occurring in the zona pellucida upon cortical reaction.

Methods Employed

For demonstration and localization of peroxidative activity a histochemical procedure was used that utilizes 3,3'-diaminobenzidine (DAB) as a substrate. Cortical reaction was initiated in cumulus-free mouse oocytes with the calcium ionophore A23187.

To confirm the specificity of our histochemical observation the peroxidase reaction was tested in the presence of two compounds that are known to inhibit the peroxidase reaction. In these control groups the incubation medium contained either phenylhydroxine or sodium sulfite.

Following the DAB reaction the oocytes were washed in 0.05 N Tris buffer, then postfixed in 1% O_sO_4 in 0.1 M cacodylate buffer then rapidly dehydrated in a series of increased concentrations of alcohol, and passed through a 2:1, 1:1, 1:2 mixtures of ethanol and Epon 812, respectively.

In separate experiments the process of zona hardening was examined in activated eggs, using the pronase bioassay. Each group of cells was observed at one minute intervals and the number of zona lysed at each time was recorded. Zona were considered lysed when they were broken at any point, or if they disappeared completely. The difference in lysis times for activated and unactivated eggs was compared using Student's paired t-test. This calculation was based on comparing the t_{50}, i.e. time required for removal of 50% of the zona pellucidae.

Major Findings

A mammalian ovoperoxidase activity was detected in ionophore activated mouse eggs. A positive DAB reaction was detected in a portion of the intact cortical granules of untreated or DMSO treated control eggs. In the ionophore activated eggs, the DAB reaction product was routinely detected by electron microscopy predominantly on the cell surface, that is, on the zona pellucida, in the perivitelline space, and in association with the cortical granule exudate. Furthermore, the peroxidase inhibitors phenylhydrazine and sodium sulfite prevented DAB staining in ionophore activated oocytes. These results indicate the presence of an ovoperoxidase, possibly of cortical granule

origin, on the surface of activated mammalian eggs, detectable by histochemical means.

Several lines of evidence indicates that the ovoperoxidase, which is activated and/or secreted from mouse eggs following artificial activation, cross-links tyrosine residues in the zona pellucida and results in hardening of the zona. Firstly, zona hardening, as determined by decreased solubility of the zona in pronase, is prevented by several compounds known to inhibit peroxidases. Inhibitors of hardening include: phenylhydrazine, sodium sulfite, sodium azide, and glycine ethyl ester. Secondly, tyrosine analogs inhibit zona hardening, unless the phenolic hydroxyl group or ortho position is blocked. That is, O-methyltyrosine (methyl substitution of phenolic hydroxyl) does not inhibit hardening; O-methyltyrosine (methyl substitution of one ortho position) partially inhibits, whereas tyramine and N-acetyltyrosine (free hydroxyl and ortho positions) effectively block hardening. Finally, exogenous horseradish peroxidase promotes limited hardening of the zona in unactivated eggs. These results are consistent with a peroxidase catalyzed cross-linking of tyrosines in the zona which results in hardening of the zona pellucida.

Significance

The secretory products of the cortical granules are responsible for changes that occur in the zona pellucida upon fertilization. Two such changes are 1) zona reaction, that is a block to polyspermy and 2) zona hardening, an increased resistance of the zona pellucida to dissolution and enzymatic treatments. Our results indicate that an ovoperoxidase is released from mouse cortical granules at activation. Furthermore, the secreted ovoperoxidase apparently catalyzes the hardening of the zona following cortical reaction. The results are consistent with an ovoperoxidase-catalyzed cross linking of tyrosines between proteins within the zona pellucida, which results in hardening.

Proposed Course

The initial interaction between sperm and egg during fertilization may be viewed as a highly specialized case of intercellular recognition. Both the zona pellucida and some fraction of the spermatozoa are believed to have equal role in the recognition and binding process, and for the most part it is thought to be species specific. In future work we shall concentrate on identification, isolation and characterization of the zona or sperm components (receptors) involved in sperm-egg binding in mammals. The program's longterm goal is to understand the mechanism(s) involved in species specific, monospermic fertilization of mammalian eggs. Specifically, (1) we shall characterize sperm cell surface antigens and identify their role in sperm binding; (2) prepare antibodies to zona specific antigens and examine their function in fertilization of subhuman primates and other animal models.

Publications

1. Gulyas, B.J.: Cortical granules of mammalian eggs. Int. Rev. Cytol., 63: 357-392, 1980.

2. Gulyas, B.J. and E.D. Schmell: Ovoperoxidase activity in ionophore treated mouse eggs: I. Electron microscopic localization. Gamete Res. 3 (September) 1980.

3. Schmell, E.D. and B.J. Gulyas: Ovoperoxidase activity in ionophore treated mouse eggs: II. Evidence for the enzyme's role in hardening the zona pellucida. Gamete Res., 3 (September) 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00029-06 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

In Vitro Studies on Primate Luteal Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	B.J. Gulyas	Senior Investigator	PRB	NICHD
Other:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
	L.C. Yuan	Chemist-Technician	PRB	NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro studies on monkey luteal cell function were further extended. Dissociated luteal cells from monkey corpus luteum were studied utilizing either short-term (3h) or long term (10d) cell culture systems. Short term studies have shown that the integrity of LH/hCG receptors is maintained after 10 min exposure to trypsin. Long-term studies have indicated that steady high levels of progesterone synthesis can not be maintained by luteal cells even when the culture medium is augmented with steroid precursor or other nongonadotropic hormones. Although under experimental conditions the endogenous estrogen levels can be suppressed, it has no effects on the declining patterns of progesterone synthesis.

Project Description ZO1 HD 00029-06 PR

In vitro studies on dissociated monkey luteal cells were further extended this year. Presently, the cellular processes which either cause or permit spontaneous regression of the CL of the menstrual cycle are incompletely understood. To facilitate studies on the cellular mechanisms that regulate luteal function we employed a long term cell culture system adaptable for monkey luteal cells. Our objective was to define those parameters that are essential in the maintenance of high levels of progesterone (P) by luteal cells cultured in vitro. Specifically: 1) examine trypsin sensitivity of LH/hCG receptors, 2) further improve the cell culture system whereby their steroidogenic activity is maintained at a steady high level.

Methods Employed

Although trypsin (TR) and other proteolytic enzymes may promote more complete dispersion of tissues, the hormone receptors of endocrine target tissues are potentially susceptible to degradation by trypsin or other proteolytic enzymes. In an attempt to justify use of TR to achieve a more thorough dispersion of luteal cell clumps, P production by dispersed monkey luteal cells from mid-luteal phase CL was examined in vitro after exposure to TR.

Corpora lutea were removed from rhesus monkeys (Macaca mulatta) at laparotomy 17-19 days after onset of menses. Luteal cells were isolated following collagenase digestion employing previously described procedures with one minor modification. Washed dissociated cells were resuspended in F10 medium containing 2% bovine serum albumin and 50μg/ml DNase. The dispersed luteal cells were filtered through a 70μm Nitex screen prior to the incubation studies.

In the first experiment, as a last step of the cell dissociation procedure, the mixtures of single cells and cell clusters were incubated in the presence of TR in F10 medium for 10 min (pre-incubation). Simultaneously, control groups were pre-incubated in F10 medium alone. At the end of pre-incubation period, cells were washed free of the pre-incubation medium and were incubated for 3 h with or without STI and/or hCG. As a control, cells were incubated in F10 medium alone. In another experiment the TR concentration was varied during the pre-incubation period. In the third experiment luteal cells were incubated continuously (3 h) at 37°C in the presence of TR alone or TR + hCG.

The last (fourth) experiment was designed to answer the question whether TR, during the continuous treatment, affects the cell receptors for LH/hCG or decreases the biopotency of hCG by hormone digestion.

In another group of experiments dissociated luteal cells were plated (5×10^4 cells/ml) and cultured in media with appropriate composition for 10 days. The basic culture medium consisted of F10 and 10% fetal calf serum (FCS). This medium was further supplemented with: 1.0 mU/ml insulin (I); 10^{-7} M thyroxin (T_4); 0.01 μg/ml cortisol (C); and/or 0.1 mg/ml cholesterol (Ch) in 0.01% ethanol, either in the presence or absence of 100 ng/ml human

chorionic gonadotropin (hCG, CR 119). Concentrations of P and estradiol (E_2) were determined in spent medium.

Lastly, we examined whether endogenous estrogens have a direct effect on P synthesis by luteal cells in culture. A known in vitro inhibitor of estrogen biosynthesis, androsta-1,4,6-triene-3,17-dione (ASD), was included in the culture medium (100 ng/ml) with or without hCG. In addition, a new compound testolactone (TESLAC), was also tested for its possible anti estrogenic properties.

Major Findings:

Pre-incubation of luteal cells with TR (first experiment with TR) had no effect on the level of P production under basal conditions. Cells that were preincubated with TR responded to hCG stimulation with increased progesterone secretion ($P<0.01$) in a fashion similar to untreated cells. P production in response to hCG was independent of TR concentration over the range of 0.05% to 0.2% during the pre-incubation period. However continuous exposure (3 h) of cells to TR significantly depressed ($P<0.01$) basal P secretion and inhibited the response to hCG. We conclude that TR had no effect on the biopotency of hCG per se, but probably the overexposure to TR had an adverse effect on the LH/hCG receptors. Addition of STI after a 10 min pre-incubation with TR, prevented these deleterious effects, thereby permitting the use of TR to improve the completeness of luteal cell dissociation.

In the long term (10 day) studies P synthesis by luteal cells in the basic medium progressively declined throughout the 10-day culture period. Although we were unable to stabilize P production under any condition tried, with the addition of I-T_4-C and in the presence of hCG, P synthesis was significantly higher ($P<0.05$) throughout the 10-day incubation period. E_2 production showed a similar declining pattern, regardless of the composition of the culture medium. Ch alone had no significant beneficial effects on maintenance of high levels of P secretion; whereas, in the presence of hCG production of P was enhanced ($P<0.01$) for the first two days of culture. In an additional experiment, luteal cells were initially cultured with I-T_4-C for 2, 4, 6 or 8 days, at which times hCG was added for the remaining days of culture. When hCG was initially added on days 2 or 4, P production was significantly elevated on days 4 and 6, and 6, respectively followed by a gradual decline. The results demonstrate that the combination of nongonadotropin hormones, when in concert with hCG, are beneficial in maintaining P synthesis at higher levels for longer intervals. Addition of hCG slowed the declining pattern of P production when introduced on culture days 2 or 4; nevertheless, the declining pattern of P output continued.

In the long term studies with the addition of ASD we demonstrated that ASD totally blocked hCG stimulated E_2 production during the first two days in culture. Thereafter, hCG failed to stimulate E_2 synthesis because E_2 levels with hCG were not different from those in control groups (F10 + FCS). Days 2-6, particularly between 4 and 6, E_2 production was slightly enhanced in the presence of hCG and ASD, as compared to any of the other groups. Although

ASD blocked hCG stimulated E_2 production during the first two days in culture, the P levels remained unaffected. During the subsequent days P levels showed gradually declining patterns. TESLAC did not block hCG stimulated E_2 production, instead, it seemed to enhance E_2 production with little or no effect on P synthesis.

Significance

The results indicate that: 1) limited exposure of monkey luteal cells to TR digestion to accomplish more complete dispersion of cells does not destroy their functional integrity or ability to respond to exogenous gonadotropin. 2) In the long term studies, additon of nongonadotropic hormones or Ch along with hCG slowed the declining pattern of P production, but the declining pattern continued. Thus, the conditions employed in our culture system do not permit imitation of the chorionic gonadotropin induced "rescue" of the CL that occurs in the fertile menstrual cycle; 3) endogenous E_2 levels may not be the cause of the declining patterns of P secretion by luteal cell in culture.

Proposed Course

Concomitant with the decline in steroidogenic activity of monkey luteal cells during culture, well defined morphological changes occur. These cell shape related changes of monkey luteal cells in culture shall be examined at the SEM and TEM levels.

Publications

1. Gulyas, B.J., L.C. Yuan and G.D. Hodgen: Progesterone production by dispersed monkey (Macaca mulatta) luteal cells after exposure to trypsin. Steroids, 35: 43-51, 1980.

2. Gulyas, B.J., L.C. Yuan and G.D. Hodgen: Synthesis of progesterone and estradiol by monkey luteal cell in culture: Effects of insulin, thyroxin, cortisol, and cholesterol with and without hCG. Biol. Reprod., 23 (October) 1980.

3. Gulyas, B.J. and G.D. Hodgen: In vitro studies on luteolysis in the rhesus monkey. In: "Dynamics of Ovarian Function", Ovary Workshop 1980 (Submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00031-07 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Hormonal Interrelationships Between Fetus, Mother and Placenta in Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	R.F. Williams	Staff Fellow	PRB	NICHD
	M.L. Yonekura	Guest Worker	PRB	NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Over the past eight years, a continuum of studies has been directed at understanding the hormonal interrelationships between the fetal, placental and maternal compartments. Most of these studies have utilized primate models, where the similarities to equivalent events in human pregnancies are most extensive. During previous years, we concluded a series of experiments which employed fetal hypophysectomy in rhesus monkeys to establish the importance of fetal hypophysical secretion for gonadal development in utero; particular attention was given to ovarian and testicular development.

During the past year, we have evaluated the development of GnRH responsiveness as a function of fetal age and sex.

Project Description: Z01 HD 00031-07 PR

Objectives

In new, but related investigations we have: 1) begun to characterize the evolution of fetal gonadal receptors for LH/hCG using florcein-labelled hCG; 2) analyzed fetal response to GnRH as a function of fetal age; 3) used ligation of transplacental vessels to induce intrauterine growth retardation, and 5) measured prolactin in maternal and placental blood after fetectomy to determine the primary origin of this hormone in the mid and late gestational milieu.

Major Findings

We conclude that a dynamic interaction of fetal and maternal hypothalamic-pituitary axes governs the development of the fetus and its endocrine organs. Survival of gametes within the fetal ovaries and testes of these primates is dependent primarily on secretions of the fetal pituitary. Current results suggest that the monkey fetus responds to GnRH injection with increased secretion of FSH and LH only during the perinatal interval in utero. Interestingly, both fetal and neonatal monkeys manifest higher biassayable LH than radioimmunoassayable LH.

We have evidence that the placental reserve is directly related to intrauterine growth retardation and is manifest after a progressive loss of functional placental tissue.

Proposed Course

Having learned that secretions of the fetal pituitary are essential to normal growth and development of ovaries and testes in utero in rhesus monkeys, we now set out to examine the secretory capacities of the fetal and newborn monkey hypophysis. Specifically, we will begin by characterization of FSH and LH concentrations in fetal and infant serum at various "ages." Tests are being conducted to examine both negative and positive feedback conditions which regulate gonadotropin secretion in utero.

Current studies will show the evolution of fetal gonadal receptors for LH/hCG during organogenesis. Also, injections of GnRH agonists and antagonists will be used to examine functional dynamics of the hypothalamic-pituitary-gonadal axis at various gestational ages. Finally, studies to monitor fetal stress acutely after exceeding the placental reserve will lead us toward early diagnosis of intrauterine growth retardation.

Publications:

1. diZerega, G. and Hodgen, G.D.: The Changing Functional Status of the Monkey Corpus Luteum. Biol. Reprod. 23: 000-000, 1980.

2. Williams, R.F. and Hodgen G.D.: Reinitiation of the Diurnal Rhythm of Prolactin Secretion in Postpartum Rhesus Monkeys. Biol. Reprod. 23: 000-000, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00045-05 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of Folliculogenesis in the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Gary D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	Wilbert E. Nixon	Senior Investigator	PRB	NICHD
	Robert F. Williams	Staff Fellow	PRB	NICHD
	Gere S. diZerega	Clinical Associate	PRB	NICHD
	Edward L. Marut	Clinical Assoicate	PRB	NICHD
	Bryan B. Cowan	Guest Worker	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Endocrine factors contributing to the regulation of folliculogenesis (selection of the dominant follicle) during the primate ovarian cycle were examined in two non-human primate models, the rhesus monkey, and the cynomolgus monkey. Circulating patterns of gonadotropins and ovarian steroids (estradiol and progesterone) were monitored by radioimmunoassay before and after follicle cautery, luteectomy and hemiovariectomy during the menstrual cycle in monkeys. In addition to examining follicle growth during the nonfertile menstrual cycle, the resumption of follicle growth was studied during the puerperium. Ovary conceptus interrelationships, as well as the cessation of follicle growth in pregnancy were studied during the fertile menstrual cycle. Salient findings include: 1) usefulness of the cynomolgus monkey as alternative non-human primate model, 2) asymmetrical function of the two ovaries during the menstrual cycle, 3) a dual stimulatory/inhibitory role for macaque chorionic gonadotropin, 4) evidence that the dominant follicle is selected during the first week of the menstrual cycle, and 5) evidence that FSH secretion is regulated by an ovarian factor(s) other than estradiol or progesterone.

Project Description ZO1 HD 00045-05 PR

Objectives

To examine the regulation of follicle growth during the primate ovarian cycle, its cessation during pregnancy, and its resumption post partum, using non-human primate models. To examine functional capacities of the hypothalamic-pituitary-ovarian axis in neonates.

Methods

Circulating patterns of gonadotropins and ovarian steroids were determined by radioimmunoassay before and after ablation of the cyclic structure (dominant follicle, or corpus luteum), or one ovary (hemiovariectomy), performed at laparotomy. GnRH challenges, castrates and steroid therapy of neonates has been undertaken. Also, adult cycling monkeys have been stimulated with hMG and clomid to examine the role of gonadotropins in follicle recruitment and selection.

Major Findings

In the primate ovarian cycle the follicle destined to ovulate in a given menstrual cycle is selected already by the mid-follicular phase, and at the expense of any other follicles of the original lot. The time course of new follicle growth after removal of the dominant follicle is unaffected by the concurrent removal of one ovary. The next follicle destined to ovulate was not selected until after the removal of the reigning dominant follicle. In the presence of the dominant follicle, the contralateral ovary contributes little, if at all, to the regulation of follicle growth, ovulation, corpus luteum function, or gonadotropin (its ablation goes "unnoticed"). Unlike bilateral ovulators, the occurrence of a "compensatory" rise in gonadotropin release after unilateral ovariectomy in this primate is conditional as the ovary removed and the surety of the dominant follicle.

Follicle growth is arrested during the luteal phase, and the follicle growth culminating in ovulation is unaffected by the concurrent removal of one ovary. The next follicle destined to ovulate is not selected until after the removal (demise) of the corpus luteum. In the presence of the corpus luteum, the contralateral ovary contributes little, if at all, to the regulation of gonadotropin secretion, follicle growth, or corpus luteum function (i.e., its removal goes "unnoticed"). Unlike bilateral ovulators, the monkey uses its ovaries in sequence, rather than in concert, and a compensatory response to unilateral ovariectomy during the luteal phase is conditional on the ovary removed, and the surety of the corpus luteum.

Constraining ovulation and corpus luteum function to one ovary has no disruptive effects on folliculogenesis. Thus, the corpus luteum, after spontaneous involution, has no local, residual effects inimicable to new follicle growth.

Two potential determinants of the resumption of folliculogenesis post partum, the rejuvenated corpus luteum, and the tonic supply of gonadotropins,

were examined in nonsuckling monkeys during the puerperium. Luteectomy at term did not advance the first ovulations post partum circulating levels of gonadotropins were similar to levels observed during pregravidic ovulatory cycles. Thus, some other factor(s) apart from the puerperal corpus luteum, or the tonic supply of gonadotropins, contribute to the regulation of folliculogenesis post partum.

Luteectomy in chronically hemiovariectomized rhesus monkeys was followed by a major increase in serum FSH, but the time-course of new follicle growth was unaffected compared to monkeys bearing two ovaries; no change in FSH levels occurred in monkeys with two ovaries, even though the opposite ovary was wedge-resected at luteectomy. Thus, following luteectomy (but not before), the ovaries may work in concert to regulate gonadotropin secretion, but the contralateral ovary (even after wedge-resection) makes the preponderant contribution. An ovarian factor other than estradiol of progesterone made a major contribution to regulating the secretion of FSH after luteectomy. In addition, folliculogenesis and the negative-feedback regulation of gonadotropins by the ovary appear to the parallel, dissociable activities.

That pregnancy creates a natural hiatus in folliculogenesis is well established. Whether this involves ovarian refractoriness to gonadotropic stimulation or suppression of hypothalamic-pituitary function has been debated at length. Our findings, using hMG/hCG therapy during mid-gestation, post partum, after early abortion and during the spontaneous luteal phase all indicate that the ovaries retain responsiveness to moderate levels of FSH and LH. We conclude that the hormonal milieu of pregnancy blocks folliculogenesis at some point(s) in the hypothalamic-pituitary system.

Among neonatal female monkeys, we found that GnRH caused an abrupt rise in FSH secretion, but a lesser increase in LH release. By 6 months of age, exogenous GnRH was barely able to effect any increase in FSH or LH. Preliminary results with the RICT in vitro bioassay indicate that the bio vs. immuno potencies of serum LH are discrepant, emphasizing the danger of relying exclusively, on RIA hormonal values.

When either ovary was removed 4 days after luteectomy the next LH surge was delayed by 4 days relative to luteectomy alone. Thus the dominant follicle is chosen after the onset of menses, but before Day 8 of the cycle.

Significance to Biomedical Research

The determination of the time of the selection of the dominant follicle (to within a week), and the elucidation of the asymmetrical activities of the ovaries during the menstrual cycle will be of vital concern to clinicans working to enhance (or suppress) fertility in women.

Proposed Course

When induction of ovulation is important clinically, two major problems are now accessable: 1) how to avoid multiple ovulations during clomid or

hMG therapy and the inherent risk in such pregnancies and 2) how to avoid premature follicular or instititial luteinization from excessive LH treatment in the follicular phase. The result is high serum progesterone and refractoriness to gonadotropins. These problems will receive intense interest in the coming year.

Publications

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2. Wilks, J.W., Marciniak, R.D., Hildebrand, D.L. and Hodgen, G.D.: Periovulatory Endocrine Events in the Stumptailed Monkey (Macaca arctoides) Endocrinology.

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7. diZerega, G.S., Marut, E.L., Turner, C.K., and Hodgen, G.D.: Asymmetrical Ovarian Function During Recruitment and Selection of the Dominant Follicle in the Menstrual Cycle of the Rhesus Monkey. J. Clin. Endocrinol. Metab., 1980.

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9. Stouffer, R. and Hodgen, G.: Induction of Luteal Phase Defects in Rhesus Monkey by Follicular Fluid Administration at the Onset of the Menstrual Cycle. J. Clin. Endocrinol. Metab., 1980.

10. diZerega, G.S., and Hodgen, G.D.: Cessation of Folliculogenesis During the Primate Luteal Phase. J. Clin. Endocrinol. Metab. 51: 158-160, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00168-04 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Ovarian Polycyclic Aromatic Hydrocarbon Metabolism, Reproductive and Oocyte Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald R. Mattison	Senior Investigator	PRB	NICHD
Other:	Maria Nightingale	Chemist	PRB	NICHD
	Kenji Shiromizu	Visiting Fellow	PRB	NICHD
	Haydeh Payami	Student Scientist	PRB	NICHD
	Lucy Chang	Biological Aide	PRB	NICHD
	Gary Hodgen	Chief (Acting)	PRB	NICHD

COOPERATING UNITS (if any) Nathanial White, Statistician, NICHD; William Hobson, Director, Int'l Center of Environ. Safety; Bal Diwan, Principle Scientist, Meloy Labs; David Longfellow, Asst. Chief CPCB, NCI; Carol Henry, Director, Dept. Exptl. Oncology, Microbiological Associates

LAB/BRANCH
Pregnancy Research Branch

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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Certain polycyclic aromatic hydrocarbons destroy oocytes in rodent ovaries. The mechanism of oocyte toxicity appears to depend upon ovarian metabolism of the polycyclic aromatic hydrocarbons to reactive intermediates which destroy oocytes. Experiments exploring the role of metabolic activation suggest that metabolism determines the rate of ovotoxivity but not the extent of oocyte destruction which occurs. The metabolic pathway to the proximate ovotoxin(s) is being explored using authentic metabolites of the polycyclic aromatic hydrocarbons. The role of ovarian and extra-ovarian metabolism in ovotoxicity is being explored. The role of detoxification in determination of the extent of oocyte destruction is being investigated. The effect of polycyclic aromatic hydrocarbons on reproductive performance of rodents is under investigation. The sensitivity of non-human primates to oocyte destruction by polycyclic aromatic hydrocarbons is being explored.

PHS-6040

Project Description ZO1 HD 00168-04 PR

Objectives

Polycyclic aromatic hydrocarbons are ubiquitous environmental pollutants. Some of these compounds are toxic to the ovaries of experimental animals, destroying oocytes, and may also impair reproduction. The sensitivity of non-human primates or humans to ovarian toxicity from the polycyclic aromatic hydrocarbons is unknown.

The three prototype polycyclic aromatic hydrocarbons studied are: benzo-(a)pyrene, 3-methylcholanthrene, and 7,12-dimethylbenz(a)anthracene. Authentic metabolites of these, provided by NCI, are also being studied.

The goal of this research is to elucidate the mechanism(s) and sites(s) of action of the ovotoxic polycyclic aromatic hydrocarbons in rodents, and non-human primates.

Major Findings

The three prototype polycyclic hydrocarbons have previously been demonstrated to destroy primordial oocytes in mice in a time, dose, strain, and metabolism dependent manner. Complete characterization of the time and dose response curves for oocyte destruction demonstrated that strain differences in metabolic activation as measured by the aryl hydrocarbon hydroxylase assay, as well as high pressure liquid chromatography, determine the rate of oocyte destruction but not the ultimate extent of oocyte destruction.

Further experiments designed to explore the relationship between metabolic activation and rate and extent of oocyte destruction also support the notion that factors other than rate of metabolic activation determine the extent of oocyte destruction which occurs after treatment with polycyclic aromatic hydrocarbons. A survey of ovarian aryl hydrocarbon hydroxylase activity and the extent of oocyte destruction in eleven inbred mice strains and two F_1 heterozygote crosses failed to demonstrate a relationship between the rate of ovarian metabolism and extent of oocyte destruction. Treatment of mice with 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent inducer of aryl hydrocarbon hydroxylase activity, did not alter the rate or extent of oocyte destruction by the three prototype polycyclic hydrocarbons tested.

Therefore, although metabolic activation of the parent polycyclic aromatic hydrocarbon is necessary for oocyte destruction to occur, other factors control the extent of ovotoxicity which occurs. Recent investigations suggest that pathways for detoxification may be crucial in determining the extent of oocyte destruction occurring after treatment with an ovotoxin. As mice mature from birth to sexual maturity, the sensitivity to oocyte destruction decreases. Over this same time period there is an increase in both ovarian glutathione and glutathione transferase. Suggesting that ovarian glutathione may play a major role in modulating ovotoxicity.

The pathways for formation of the proximate ovotoxin are not known. Recent experiments demonstrate that the 3-phenol, 4,5-oxide, and both isomeric

forms of the 4,5-dihydrodiol of benzo(a)pyrene are less ovotoxic than the parent hydrocarbon. This suggests that the monooxygenases active in metabolizing benzo(a)pyrene in the 3,4,5,-region are not responsible for the formation of the ovotoxic metabolites.

The role of ovarian and extra-ovarian metabolism of the parent polycyclic aromatic hydrocarbon to the ovotoxic metabolite is unknown. Experiments utilizing intra-ovarian injections of small volumes of polycyclic aromatic hydrocarbons demonstrates oocyte destruction. These experiments suggest that the ovary is fully capable of activating polycyclic aromatic hydrocarbons to ovotoxic metabolite(s). These experiments, however, do not exclude the possibility that extra-ovarian metabolism may also contribute to the oocyte destruction which occurs when an organism is exposed to an ovotoxin.

Although oocyte destruction occurs after treatment with polycyclic aromatic hydrocarbons, additional reproductive toxicity is also possible. Breeding experiments suggest that polycyclic aromatic hydrocarbons may decrease fertility in the presence of sufficient stores of oocytes, and without altering ovarian response to gonadotropins.

Preliminary experiments suggest that the ovaries of non-human primates are also susceptable to adverse effects after treatment with polycyclic aromatic hydrocarbons.

Significance to Biomedical Research and the Program of the Institute

Reproduction does not take place in a chemically pristine environment. Understanding the mechanism(s) of action, site(s) of toxicity, and species susceptibility to reproductive toxins is necessary in determining risks to human reproduction. These experiments provide the basis for elucidation of the mechanism of action of one class of reproductive toxins and will therefore increase our understanding of reproductive toxicology.

Proposed Course

Further characterization of the proximate ovotoxin, role of detoxification and ovarian and extra-ovarian metabolism is planned. The reproductive performance studies will be completed during the coming year.

A major technical problem in this research is the method of oocyte analysis which requires serial sectioning of ovaries and manual counting. Further research is planned to explore the development of automated methods for oocyte counting using flow cytometry.

Further characterization of the sensitivity of non-human primates to reproductive toxicity after treatment with polycyclic aromatic hydrocarbons will continue.

Publications

1. Mattison, D.R.: How xenobiotic compounds can destroy oocytes. Contemporary OB/GYN. 15, 157-169, 1980

2. Mattison, D.R. and Thorgeirsson, S.S.: Ovarian aryl hydrocarbon hydroxylase activity and primordial oocyte toxicity of polycyclic aromatic hydrocarbons in mice. Cancer Res. 39, 3471-3475, 1979.

3. Mattison, D.R., West D.M., and Menard, R.H.: Differences in benzo(a)pyrene metabolic profiles in rat and mouse ovary. Biochem. Pharmac. 28, 2101-2104, 1979.

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5. Mattison, D.R.: Morphology of oocyte and follicle destruction by polycyclic aromatic hydrocarbons in mice. Toxicol. Appl. Pharmacol. 53, 249-259, 1980.

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7. Mattison, D.R.: The effects of biologically foreign compounds on reproduction. In Drugs in Pregnancy, Ed. R.W. Abdul-Karim. In Press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00900-02 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Factors Regulating Estrogen and Progesterone Receptors in Monkey Endometrium in the Fertile Menstrual Cycle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G. D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	B. Kreitmann	Guest Worker	PRB	NICHD
	O. Kreitmann	Guest Worker	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Appropriate methods for repeated surgical collection of endometrial tissue from monkeys and characterization of cytosol and nuclear estrogen and progesterone receptors have been developed. Equilibrium dissociation constants of estradiol and progesterone were 2.1×10^{-10}M and 3.6×10^{-9}M, respectively. When estrogen or estrogen + progesterone replacement therapy was given to castrate monkeys, we found that progesterone receptor synthesis was induced by estrogens.

Project Description ZO1 HD 00900-02 PR

Objectives

To develop surgical and other methodologic procedures for studying factors influencing endometrial receptors for estrogens and progesterone in monkeys. To investigate the action of these receptors in facilitating implantation. To exploit these findings for the purposes of fertility regulation, either to resolve infertility or provide contraception.

Methods

At abdominal transfundal hysterotomy, we were able to collect endometrial tissue repeatedly. Standardized techniques used characterization of steroid receptors in other species were adapted to optimal circumstances. By giving estrogen and progesterone replacement therapy to castrate monkeys in a pattern which mimicks the normal ovulatory menstrual cycle, follicular phase and luteal phase factors influencing receptor formation were evaluated.

Major Findings

Simulated menstrual cycles were produced in 20 castrate monkeys by sequential treatment with estradiol and progesterone in silastic capsules. RIA of E_2 and P, and gonadotropins in peripheral serum provided assuredness of the hormonal status of each monkey under treatment. Cytosol and nuclear receptors for E_2 and P were measured in the endometrium after different intervals of the treatment. E_2 receptor (E_2R) levels were not changed during the estrogen sequence, but were lowered by progesterone therapy in both cytosol and nuclear components. Progesterone receptor (PR) synthesis in cytosol was induced by exogenous estrogen. The total concentration of PR decreased with the uptake of P by the cell; meanwhile, the ratio of cytosol to nuclear P receptors declined. These data suggest that this sequential estrogen-progesterone regimen induces the changes in E_2R and PR patterns in endometrium of ovariectomized monkeys which occurs due to ovarian cyclicity in the normal menstrual cycle.

Proposed Course

Current studies center on the changes in endometrial steroid receptors that acompany implantation in the fertile menstrual cycle and the abnormal characteristics of endometrial receptors for these steroids during short luteal phase infertility.

References

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2. Kreitmann-Gimbal, B., Goodman, A.L., Bayard, F. and Hodgen, G.D.: Characterization of Estrogen and Progesterone Receptors in Monkey Endometrium: Surgical and Biochemical Methodology and Effects of Estradiol and/or Progesterone on Castrate Monkeys. Steroids 34: 749-770, 1979.

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4. Kreitmann, O., Bayard, F. and Hodgen G.D.: 17β-hydroxysteroid dehydrogenase in monkey endometrium during the menstrual cycle and at the time of implantation. Steroids (in press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00901-02 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Core Endocrine Assays Laboratory

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	W.E. Nixon	Senior Investigator	PRB	NICHD
Other:	R. Reid	Biol. Lab. Tech.	PRB	NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Collaboration with and support of numerous NICHD programs in areas where required specialized procedures are unavailable elsewhere, or cannot be obtained in a timely manner continues as a high priority consideration. Critical reagents are supplied at large savings compared to commercial rates. Laboratory training for clinical and research associates is provided as required.

Collaborative studies of ovarian function in the monkey have assessed the comparative values of ovulation induction agents, pergonal, neopergonal, clomiphene, etc., on folliculogenesis as opposed to spontaneous development and ovulation. This investigation seeks to enhance ova production for use in several strategies to accomplish in vitro fertilization in this model system.

Core Endocrine Assays Laboratory

The rapid estradiol radioimmunoassay (up to 30-35 serums received and results reported daily) continues to consume much laboratory time and effort. Two major Pregnancy Research Branch projects, ovum collection for investigation of alternative implantation strategies and an immunologic intervention in sperm-egg interactions, are dependent upon the daily assessment of the monkey's reproductive endocrine milieu for making critical decisions involving surgery among the many protocol requirements. Clinical applications involving carefully timed hormonal induction of ovulation were also supported. Observations at laparoscopy in monkeys, cervical mucus characteristics in women and extended serum steroid analysis support the appropriateness of this assay in predicting the ovulatory period in both women and monkeys.

Steroid radioimmunoassays for progesterone, testosterone, androstenedione, estradiol and estrone have been established and utilized in support of NICHD porgrams. Gonadal function in monkeys and rodents has been studied, and reagents have been made available to members of the scientific community outside the NIH.

The highly specific hCGβ RIA for chorionic gonadotropin continues to be utilized for clinical and research applications.

The cyclic nucleotides (cAMP & cGMP) RIA methodologies have been established in the laboratory and subjected to limited application.

Production and distribution of sheep anti-rabbit gamma globulin serum (2nd antibody) for use in NICHD programs with a replacement cost in excess of $200,000 is an on-going project which yields a product with high titer and quality and immediate availability.

Clinical and research associates who join the Pregnancy Research Branch often have no experience with polypeptide or steroid hormone RIA. During a specified interval of exposure to theoretical considerations, observation and hands-on experience, they become acquainted with RIA procedures. This familiarization includes reagent requirements, assay validity, control requirements, data analysis and supplemental procedures as required to enhance RIA (extraction, chromatography, etc.). Thus, many become familiar with one of the data acquisition processes that may be vital in their patient care and/or research activities.

In collaborations within PRB, monkey corpora lutea are expected to become available during the cycling season for this animal. Further studies on corpus luteum function as expressed by progesterone secretion by dispersed cells from various phases of the menstrual cycle will be investigated. The effect of administered agents (hCG, etc.) affecting reproductive function in this animal will be assessed also.

The corpus luteum is primarily a progesterone secreting organ, however, Stouffer et al. have reported estrogen production also. Further studies of androgen and estrogen production are planned.

The rapid estrogen radioimmunoassay for predicting the ovulatory period in monkeys will be used in conjunction with studies of ova collection and implantation as well as studies of menstsrual cycle events of the peri-ovulatory period. It is anticipated that much laboratory time as well as substantial resources will be utilized, however, the contribution to NICHD programs will be invaluable.

The training function for Clinical and Research Associates will be further systematized with regard to time in the lab, the kinds of experiences provided, providing experiences based on previous background, and ensuring the appreciation of the essentials by each Associate for whom this may be a terminal exposure to laboratory methodology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00906-01 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Metabolism of Steroids and Xenobiotic Compounds by Microsomal Monooxygenases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald R. Mattison	Senior Investigator	PRB	NICHD
Other:	Maria Nightingale	Chemist	PRB	NICHD

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Matti Lang, Visiting Fellow, NICHD

LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.25	.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The microsomal cytochrome P-450 dependent monooxygenases are important in the metabolism of many steroids and xenobiotic compounds. The interaction of these compounds may alter both steroid and xenobiotic metabolism. Alteration of steroid secretion or clearance may alter reproduction. Modification of xenobiotic metabolism may enhance or diminish any toxicity produced by the biologically foreign compound.

Project Description ZO1 HD 00906-01 PR

Objectives

Many steroids important in reproduction are synthesized or cleared by enzymatic steps utilizing microsomal cytochrome P-450 dependent monooxygenases. A large number of xenobiotic compounds are also metabolized by this family of enzymes. One possible site for interference of reproduction by xenobiotic compounds may be competition for, induction, destruction, or repression of these monooxygenases. The focus of this research is to explore the interaction of steroid and xenobiotic metabolism as it impinges on reproduction.

Major Findings

Developmental, induction, and combined incubation studies demonstrate that the monooxygenases responsible for the three hydroxylation of benzo(a)-pyrene, demethylation of ethylmorpaine, and two hydroxylation of estradiol (catechol estrogen formation) are all distinct monooxygenases, independent and under different regulatory mechanisms. None of the xenobiotic compounds tested to date have any influence either in vivo or in vitro on the formation of catechol estrogens, suggesting stringent substrate specificity and enzyme regulation. The monooxygenases responsible for the hydroxylation of benzo(a)-pyrene however appear less specific, being inhibited by estradiol as well as other xenobiotic compounds.

Significance to Biomedical Research and the Program of the Institute

Previous investigations in other laboratories have demonstrated that alteration of steroid metabolism by xenobiotic compounds like the halogenated hydrocarbon pesticides can impair reproduction in rodents and may have similar effects in primates. Understanding the interactions between xenobiotic compounds and steroids with respect to microsomal metabolism may help define more clearly these mechanisms of reproductive toxicity.

Proposed Course

Further characterization of the monooxygenase(s) responsible for formation of catechol estrogens will continue. Additional experiments will characterize the effect of selected xenobiotic compounds on steroid metabolism in experimental animals.

Publications

1. Mattison, DR, Nebert, DW, and Lang, M. Multiple forms of hepatic microsomal cytochrome P-450 responsible for monooxygenation of estradiold-17 benzo(a)pyrene, acetanilide, and ethylmorphine. In, The Second International Congress on Microsomes and Drug Oxidation. ed. J.A. Gustafsson, M.J. Coon. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00907-01 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Reproductive Toxicity of Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Donald R. Mattison	Senior Investigator	PRB	NICHD
Other:	Maria Nightingale	Chemist	PRB	NICHD
	Kenji Shiromizu	Visiting Fellow	PRB	NICHD
	Gary Hodgen	Chief (Acting)	PRB	NICHD
	Donald Barber	Biological Laboratory Technician	PRB	NICHD

COOPERATING UNITS (if any) Snorri Thorgeirsson, Head, Biochem. Pharm. Sect., NCI; Anthony Fauci, Head, Clin. Physiol. Sect., Lab. Clin. Invest., Dept. Clin. Dir , NICHD; Susan Fabro, Head, Pharm. & Exper. Thera. Sect., NCI; Thomas Shawker, Chief, Ultrasonic sound, CC

LAB/BRANCH
Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.25	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Certain drugs of choice for many neoplastic and some non-neoplastic diseases produce human reproductive toxicity. Detailed studies of these patients before, during and after treatment may help elucidate the mechanisms of, and sites of reproductive toxicity. Parallel experimental studies in rodents and non-human primates will allow a more specific dissection of the mechanism of reproductive toxicity of these drugs as well as the sensitive reproductive processes.

PHS-6040

Project Description ZO1 HD 00907-01 PR

Objectives

Certain drugs used in the treatment of many neoplastic, as well as some non-neoplastic diseases impair fertility in humans. Cyclophosphamide, a widely used alkylating agent has been demonstrated to produce amenorrhea, and an age and dose dependent premature ovarian failure in women. We are exploring the mechanism of action of reproductive toxicity of cyclophosphamide in rodents, non-human primates and women. The women studied are patients at the clinical center, being treated for diseases for which cyclophosphamide is the drug of choice. The goal of this research is to elucidate mechanisms, sites of action, and species susceptability of drugs producing reproductive toxicity.

Major Findings

Studies in rodents, in collaboration with Snorri S. Thorgeirsson, NCI, (ZO1 CM 06137-02 LCHP) have demonstrated that cyclophosphamide destroys primordial oocytes in rodents in a time, and dose dependent manner. Little toxicity is seen in growing or pre-ovulatory follicles. Strain and species differences in sensitivity to oocyte destruction have been observed but are not understood at the present time.

Observations on women treated with cyclophosphamide suggest further species differences in follicle sensitivity to the drug. Women chronically treated with cyclophosphamide generally develop amenorrhea shortly after starting therapy, suggesting effects on pre-ovulatory follicles. This is in contrast to the primordial oocyte destruction occurring in rodents.

Significance to Biomedical Research and the Program of the Institute

Reproductive toxicity of drugs used in the treatment of human disease is is a significant side effect. With increasing success in producing prolonged remissions in patients with cancer, premature ovarian failure is a major concern of the patient and her family. Understanding the mechanism of reproductive toxicity of these drugs may allow the design of alternate treatment schedules, or use of different drugs to decrease this side effect. An additional gain from these studies is the knowledge of comparative mechanism of action of reproductive toxins in humans and experimental animals.

Proposed Course

Further research will explore the toxicity of cyclophosphamide in nonhuman primates to determine effects on ovarian and pituitary function, as well as follicle susceptibility. Detailed studies will continue in rodents to elucidate the mechanisms of activation, and pathways for detoxification. We will also continue our observations of patients treated with cyclophosphamide, as well as other known or suspect reproductive toxins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00908-01 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Genetics of Murine Atresia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Donald R. Mattison	Senior Investigator	PRB	NICHD
Other:	Lucy Chang	Biological Aide	PRB	NICHD

COOPERATING UNITS (if any)
Carol Henry, Director, Dept. Exptl. Oncology, Microbiological Associates

LAB/BRANCH
Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The genetics of atresia is being explored in several inbred strains of mice and their heterozygote crosses. Initial experiments have identified differences in the rates of atresia in several inbred mice strains as well as their F_1 heterozygotes.

PHS-6040

Project Description ZO1 HD 00908-01 PR

Objectives

Previous investigations in other laboratories have demonstrated differences in the rates of atresia among different inbred strains of mice. However, the genetics of these differences in rates of atresia have not been explored in detail. These experiments have been designed to elucidate genetic aspects of atresia using inbred mice and their heterozygote crosses.

Major Findings

The rate of atresia is more rapid in C57BL/6N mice than DBA/2N mice or their F_1 heterozygote cross. The rate of atresia is more rapid in C3H/AnfCum mice than C57BL/6Cum mice or their F_1 heterozygotes. DBA/2J and DBA/2N mice have similar rates of atresia, as do C57BL/6N and C67BL/6Cum mice.

Significance to Biomedical Research and the Program of the Institute

The ultimate event of reproductive senescence in female experimental animals, as well as women, is the depletion of oocytes from the ovary. The age at which the ovary is depleted of oocytes depends in part upon the initial complement of oocytes, the rate of atresia, as well as the effects of exposure to ovotoxins. Characterization of the genetics of atresia in mice may provide insight into the biological nature of the process. Additionally, these studies may also help clarify the interrelationship between oocyte toxicity and atresia.

Proposed Course

Further characterization of the rates of atresia in these inbred strains and their crosses will continue.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00909-01 PR

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of ethanol on the developing mammalian fetus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	A.B. Mukherjee	PRB	NICHD
Other:	L. Golden	PRB	NICHD
	J. Moy	PRB	NICHD
	L. Tamarkin	NPMB	NICHD

COOPERATING UNITS (if any)
Neonatal and Pediatric Medicine Branch, NICHD

LAB/BRANCH
Pregnancy Research Branch

SECTION
Molecular and Development Genetics Unit

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the last decade much data have accumulated to show that ethanol administered during intrauterine development of the fetus adversely affects the outcome of pregnancy. A new syndrome known as fetal alcohol syndrome (FAS) has been recognized. We have started a project to examine the various effects of ethanol on the developing mammalian fetus. So far we have examined the effect of ethanol on the β-endorphin receptor levels in the adult rat brain as well as the γ-glutamyl transpeptidase activity in various organs of these animals. It has been found that both of these parameters are adversely affected by ethanol. We plan to examine these parameters in the fetuses of alcohol and non alcohol treated animals.

PHS-6040

Objectives: The evidence that chronic ethanol abuse during pregnancy affects the developing fetus is substantial. Although a large amount of data is available on the teratogenic effects of ethanol, the biochemical mechanism for the development of fetal alcohol syndrome has not been understood. The other aspect of alcoholism is the higher rate of alcoholism among the children of alcoholics. The reason for this higher incidence is not known. One way to approach these problems would be to identify some parameters which identify the alcoholic individual prior to the development of this disease. Our main objective was to find out whether or not there are any biochemical parameters that could be quantitated in the fetus that would correlate with ethanol consumption by the mother during pregnancy.

Major Findings: We have evaluated two biochemical parameters so far in the adult rats: (i) β-endorphin receptor level in the brain of ethanol treated and nonfed animals; (ii) γ-glutamyl transpeptidase levels in different organs of animals fed with alcohol, and nonfed controls.

Significant increase in β-endorphin receptor level occurred in the brain of rats which were ethanol-fed. This increase was more pronounced after six days of ethanol consumption.

The effect of ethanol on γ-glutamyl transpeptidase level in various organs is very interesting. At concentration of ethanol used (100mg%) the enzymic activity increased 20 fold in the kidneys and 10 fold in the liver, but no significant increase occurred in the brain throughout the course of the experiment (21 days). This was true for both the mothers as well as the fetuses. The alcohol treated group gave birth to fetuses with lower birth weight than in the control group. The higher level of γ-glutamyl transpeptidase activity persisted for a prolonged period of time in the progeny which were allowed to live on alcohol free diet.

Significance of the results: There are no well established quantitative measures of the effect of chronic ethanol abuse. Why some people become alcoholic is not clearly understood. From the standpoint of growing alcoholism among the teenagers, any parameter that leads to identification of alcohol abusers or potential abusers would be important. The effect of ethanol on the β-endorphin receptors as well as on the γ-glutamyl transpeptidase activity may lead to the identification of such markers of alcoholism.

Proposed Course: We plan to study the effects of ethanol on β-endorphin level in the brain and blood of ethanol fed animals. Experiments will be conducted to delineate if there is a correlation between the amount of β-endorphin and the β-endorphin receptor level in chronically ethanol fed animals. Additionally, experiments are now being conducted to explore whether or not ethanol disturbs the melatonin rhythm in the pineal gland of the treated animals. These studies are being conducted in collaboration with Dr. Lawrence Tamarkin.

Publications:

1. Mukherjee, A.B. and Golden, L.: Effects of chronic ethanol feeding on β-endorphin receptor level in the rat brain (in preparation).

2. Mukherjee, A.B. and Moy, J.: Effect of ethanol on the maternal and fetal γ-glutamyl transpeptidase levels in different organs (in preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00910-01 PR

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Possible role of uteroglobin, transglutaminase and progesterone in the success of an allotransplantation in nature: mammalian pregnancy.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.B. Mukherjee	PRB	NICHD
OTHER:	A.K. Agrawal	PRB	NICHD
	L. Golden	PRB	NICHD
	R. Ulane	NPMB	NICHD
	B. Foley	PRB	NICHD

COOPERATING UNITS (if any)
K. Laki, Sec. Physical Chem., NIAMDD.

LAB/BRANCH
Pregnancy Research Branch

SECTION
Molecular and Developmental Genetics Unit

INSTITUTE AND LOCATION
PRB, NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To ascertain the immunosuppressive role of uteroglobin its effect on the incorporation of H^3-thymidine into maternal lymphocytes in a mixed culture with mitomycin-C inactivated blastomeres was assessed. It was found that when rabbit blastomeres were mixed with maternal lymphocytes, heavy incorporation of H^3-thymidine into the DNA of lymphocytes occurred suggesting maternal recognition of fetal antigens. However, such stimulation is totally suppressed when blastomeres were treated with uteroglobin and transglutaminase and then added to the lymphocyte cultures. Inhibition of transglutaminase by NPCNU or MDC failed to produce the suppressing effect of uteroglobin. There results suggest: (i) that uteroglobin may be involved in immunosuppression in the rabbit uterus during preimplantation stages; (ii) uteroglobin may crosslink with part of the H-2 antigen of the embryo, thus masking this antigen from the lymphocytes of the mother and; (iii) this crosslinking of uteroglobin and H-2 antigen may be catalyzed by the uterine enzyme transglutaminase.

Objectives: To delineate the possible role of uteroglobin, transglutaminase and progesterone in non-rejection of the mammalian fetus by the mother.

Major Findings: It was found that uteroglobin suppresses incorporation of H^3-thymidine into maternal lymphocytes when stimulated with mitomycin-C inactivated embryonic cells from the same animal. Using a physiological concentration of uteroglobin in the presence of transglutaminase suppression of embryonic antigen recognition by maternal lymphocytes has been achieved. Inhibition of transglutaminase leads to the elimination of this effect of uteroglobin. Progesterone has a synergistic effect when used in combination with uteroglobin and transglutaminase in suppressing stimulation of maternal lymphocytes by embryonic antigens.

Significance to Biomedical Research: Uteroglobin is a small molecular weight protein synthesized by endometrial cells of the rabbit upon stimulation with progesterone. This protein is stynthesized only between day 3 to 9 of pregnancy. Since its discovery, more than 10 years ago, no function of this protein is known, as yet, other than its ability to bind progesterone. Our data suggest an immunosuppressive role of this protein during early development in the rabbit. We believe, similar proteins may also be found in other mammals as well. The mechanism for non-rejection of the mammalian fetus (allograft) by the mother is not well understood. However, it seems probable that uteroglobin complexes with the H-2 (β_2-microglobulin) antigen of the embryonic cell surface. This is catalyzed by the enzyme transglutaminase. Consequently, the embryonic transplantation antigens are masked and the maternal lymphocytes fail to recognize the embryos as foreign. This finding may explain the mechanism of one of the most intriguing phenomenon in biology namely the non-rejection of a mammalian fetus by the mother during pregnancy.

Proposed Course: Uteroglobin is present in the lung and in the prostate gland in small quantities. The complete list of its organ distribution is as yet unknown. We plan to develop a radioimmunoassay using monospecific antibody to this protein produced in vitro by the hybridoma technique. We would also like to test the cross reactivity, if any, of this antibody to uterine proteins in other animals including the human. If such a protein is found its immunosuppressive properties will also be tested. The immunosuppressive properties of the lung and prostate uteroglobin will also be tested. Additionally, the temporal relationship of transglutaminase and uteroglobin during early pregnancy will also be ascertained.

Publications:

1. Mukherjee, A.B., Laki, K., and Agrawal, A.K.: Possible mechanism of success of an allotransplantation in nature: Mammalian pregnancy. Medical Hypothesis, in press.

2. Mukherjee, A.B., Ulane, R., Agrawal, A.K.: Possible role of uteroglobin transglutaminase and progesterone in the success of a natural transplantation: Mammalian pregnancy. Proceedings of the National Academy of Sciences (in preparation).

3. Mukherjee, A.B., Laki, K., Agrawal, A.K., Golden, L.: Inhibition of transglutaminase during preimplantation stages prevents pregnancy in the mouse. (in preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00911-01 PR

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Superovulation induced intrauterine growth retardation in mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.B. Mukherjee	PRB	NICHD
OTHER:	J.D. Schulman	NPMB	NICHD

COOPERATING UNITS (if any)

M. Evans, University of Chicago School of Medicine, Dept. Obs. Gyn.

LAB/BRANCH
Pregnancy Research Branch

SECTION
Molecular and Developmental Genetics Unit

INSTITUTE AND LOCATION
PRB, NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An attempt was made to develop an animal model for Intra Uterine Growth Retardation (IUGR) in the mouse. Superovulation produced approximately twice the number of embryos in the mouse and produced significant crowding and competition for maternal nutrients and oxygen. Partial hemiophorectomy was performed in some animals to attempt intra-animal comparisons. The results of these experiments suggest that superovulation may be an easy, quick and inexpensive method of achieving IUGR in mice.

Intrauterine growth retardation (IUGR) continues to be a source of significant perinatal morbidity and mortality. The etiology, pathogenesis, and predisposing factors of IUGR have yet to be satisfactorily delineated. Animal models may allow a close scrutiny of many factors in IUGR, but such models to date have been of limited use because of significant differences in pathogenesis or maternal affects.

Using the technique of superovulation we have developed a mouse model of IUGR. Three groups of animals were studied: (a) total hemioophorectomy, (b) partial (approximately 50% of one side) hemioophorectomy, and (c) no surgery. All these animals were superovulated using standard techniques. Non-superovulated animals served as controls. Total body weight of each individual fetus, the brain, liver, placental weight, and serum glucose levels in each fetus was also measured. The brain liver ratio was calculated for each group of fetuses.

Superovulation produced significant alterations in most parameters tested as compared to controls. Fetal body weight was significantly reduced by 23%, brain weight by 12%, and liver weight by 39%. Consequently, the brain liver ration which was 1.04 in controls, increased to 1.58, a situation corresponding well to human *in vivo* IUGR data. Placental weights were decreased by 34% whereas serum glucose was unaffected.

These data suggest that superovulation may be an easy, quick, and inexpensive method of achieving IUGR in mice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00912-01 PR

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Gene Regulation and Cellular Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Janice Y. Chou	Senior Investigator	PRB, NICHD
OTHER:	Toshiaki Mano	Visiting Fellow	PRB, NICHD
	Richard Staton	Technician	PRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Topics of current interest are: 1) control of the expression of human chorionic gonadotropin (hCG), pregnancy-specific β-1-glycoprotein (SP1) and alkaline phosphatase in placenta, SV40 tsA-transformed placental, choricocarcinoma, and nontrophoblastic tumor cells; 2) control of the expression of α-fetoprotein (AFP), albumin, and transferrin in liver, SV40 tsA-transformed liver, and hepatoma cells; 3) hormonal regulation of glycogenolysis and gluconeogenesis in liver.

Project Description:

Objectives: The main objectives of this project are: (1) to study cellular differentiation and gene regulation in placenta and liver; (2) to isolate and characterize selected enyzmes and other proteins from normal placenta, choriocarcinoma cells, SV40-transformed placental cells and SV40-transformed rat liver cells; and (3) to characterize the hormonal regulation of glycogenolysis and gluconeogenesis in liver.

Methods Employed: The principal methods employed are: (1) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, ion-exchange chromatography, and affinity chromatography; (2) enzyme assays, which are usually based on spectrophotometric or radiometric methods; (3) radioimmunoassays of hormones, liver proteins, and cAMP; and (4) transformation of placental cells and liver cells with SV40.

Major Findings: (1) The synthesis of hCG in the tsA-transformed placental cells was induced by sodium butyrate and 5-bromo-2'-deoxyuridine (BrdUrd) at 33°C (the temperature at which the cells have the transformed phenotype) and at 40°C (the temperature at which the temperature-sensitive tsA-transformants regain their nontransformed phenotype). The synthesis of hCGα in these transformed cells was induced by sodium butyrate and BrdUrd at 33°C but was inhibited by both agents at 40°C. The synthesis of hCG and hCGα in choriocarcinoma cells was inhibited by sodium butyrate and BrdUrd. Our data indicate that the effects of sodium butyrate and BrdUrd on the production of hCG, but not hCGα, were different in the SV40 tsA-transformed placental cells and choriocarcinoma cells. (2) Alkaline phosphatase in human placental cells transformed by tsA-mutants of SV40 can be greatly induced by growing these cells at 40°C. The induced alkaline phosphatase from a term placental transformant (TPA30-1) was purified, characterized and compared with alkaline phosphatase from term and first trimester placentas. The TPA30-1 alkaline phosphatase differs from the enzyme from term placenta but indistinguishable from the phosphatase from first trimester placenta in physiochemical and immunological properties. The induction of first trimester phosphatase in cells derived from term placenta provides a system for the study of alkaline phosphatase gene regulation in human placenta. (3) Clonal rat fetal liver cell lines that expresses the functions of differentiated liver cells under controllable conditions has been established. Normal fetal liver cells were transformed by tsA mutants of SV40. These transformed cells are temperature sensitive in maintenance of the transformed phenotype. The transformed fetal liver cells contained functional receptors for glucagon, as shown by the stimulation of intracellular cAMP accumulation by glucagon. The response to glucagon was dose dependent ($K_{act} = 5 \times 10^{-8}$ M). Addition of cortisol to the culture medium enhanced the glucagon response selectively at the restrictive temperature. (4) Using newly developed radioimmunoassay for pregnancy-specific β_1-glycoprotein (SP1), we found that the tsA-transformed placental cells synthesized low levels of SP1 at 33°C. At 40°C, SP1 production was greatly increased. Choriocarcinoma cells, on the other hand,

did not synthesize detectable amounts of SP1 in culture. Therefore, the tsA-transformed placental cells should be useful in studying SP1 synthesis in human placentas.

Significance to Biomedical Research and the Program of the Institute: The study of the mechanisms of mammalian cell differentiation is of importance because it may lead to an understanding of normal and abnormal cellular functions.

The investigations of hormonal regulation and gene expression in placenta and liver are essential for an understanding of fetal development and embryogenesis. The study of the control of gene expression of alkaline phosphatase, hCG, SP1, and AFP is of importance because these substances are derepressed in many tumors. Ectopic production of these fetal proteins may serve as an early signal of malignant transformation. The establishment of human placental cells and rat fetal liver cells which approximate normal placental and liver cells in vivo respectively should help us to elucidate the control mechanisms for the synthesis of these proteins.

Proposed Course of Research Project: (1) To study the synthesis and processing of hCG, SP1, and alkaline phosphatase in placental cells. (2) To study the synthesis and processing of α-fetoprotein, albumin, and transferrin in liver cells. (3) To study the molecular mechanisms regulating the levels of the placental and liver proteins in these conditionally transformed mammalian cells. To determine: a) whether the levels of expression of these proteins are regulated by transcription and/or; b) whether translational control is a factor in the control of gene expression. (4) Delineate the mechanism regulating the synthesis of these placental and liver proteins by hormones and other effectors. (5) Further characterize the glucagon response in liver cells.

Publications:

1. Chou, J.Y.: Regulation of the induction of alkaline phosphatase in choriocarcinoma cells by sodium butyrate. In Vitro, 15, 789-795, 1979.

2. Chou, J.Y.: Gene regulation in placenta: A model system using human placental cells transformed by tsA mutants of simian virus 40. In Frank-Gunter Lehmann (ed.): Carcino-Embryonic Proteins, Vol. 2. Elsevier/North-Holland, 1979, pp. 643-650.

3. Chou. J.Y.: Regulation of human chorionic gonadotropin synthesis in placenta, choriocarcinoma, and non-placental tumors. In Segal, S.J. (ed.). "Chorionic Gonadotropins" in Topics in Population Sciences. New York, Plenum Press, 1980, in press.

4. Schlegel-Haueter, S.E., Schlegel, W., and Chou, J.Y.: Establishment of a fetal rat liver cell line that retains differentiated liver functions. Proc. Natl. Acad. Sci., 77, 2731-2734, 1980.

5. Sakiyama, T., Mano, T., and Chou, J.Y.: Synthesis of first trimester placental alkaline phosphatase in cultured human term placental cells. J. Biol. Chem., 1980, in press.

6. Chou, J.Y.: Effects of sodium butyrate and 5-bromo-2'-deoxyuridine on the synthesis of human chorionic gonadotropin in human placental cells transformed by tsA mutants of simian virus 40. Endocrinology, 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00913-01 PR

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Primate Models for In Vitro Fertilization and Alternatives

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	O. Kreitmann	Guest Worker	PRB	NICHD
	B. Cowan	Guest Worker	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed procedures for oocyte aspiration from the preovulatory follicle, in vitro fertilization, embryo culture and an alternative low tubal ovum transfer surgical procedure allowing in vivo fertilization.

PHS-6040

Project Description Z01 HD 00913-01 PR

Objectives

Collection of the "ripe" oocyte from the preovulatory follicle is difficult to schedule appropriately. The fertilizability of the "unripe" egg is poor, but writing too long will permit spontaneous rupture of the dominant follicle and loss of the oocyte. We seek to maximize the skill of "timing" aspiration of the oocyte.

Major Findings

Aspiration of the preovulatory follicle induces luteal dysfunction, probably due to removal of granulosa cells.

We have succeeded in achieving in vitro fertilization of monkey oocytes.

After low tubal ovum transfer, we obtained 5 pregnancies after 31 attempts.

Proposed Course

Two major problems remain priority studies: 1) Development of replacement therapy regimens that correct induced luteal dysfunction after folli cular aspiration; and 2) overcoming the asynchrony between retarded embryo development in vitro and maternal uterine status at the time of embryo transfer.

Publications:

1. Kreitmann, O. and Hodgen, G.D.: Low Tubal Ovum Transfer: An Alternative to In Vitro Fertilization. Fert. Steril. (in press).

2. Kreitmann, O., Lynch, A., Nixon, W.E. and Hodgen, G.D.: Ovum Collection, Maturation and Fertilization In Vitro, and Embryo Transfer in Primates. "Embryotransfer and Instrumental Insemination" World Conference, Kiel, Germany September 24-27, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00914-01 PR

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Fallopian Tube Dysfunction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	G.S. diZerega	Clinical Associate	PRB	NICHD
	L.A. Werlin	Clinical Associate	PRB	NICHD
	A. Munabi	Guest Worker	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Pregnancy Research Branch

SECTION

Section on Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major cause of human infertility in the U.S.A. is tubal dysfunction. Endometriosis and other causes of failure to achieve satisfactory gamete transport are major contributing factors. We sought to examine how these infertilities arise and how to better treat them medicinally and surgically.

PHS-6040

Project Description: Z01 HD 00914-01 PR

Objectives

The cause of infertility when endometrial tissue grows ectopically is unknown; even its hormonal support is poorly understood. We studied: 1) the role of estrogens and progesterone in the maintenance of ectopic endometrial plaques; and 2) the effect of ectopic endometrium on ovum and sperm transport.

Major Findings

Either estrogen or progesterone, alone or in combination, support survival of endometrial plaques. Removal of steroids suppresses long existing ectopic endometrium within 4 to 6 weeks.

Proposed course

Current projects are aimed at determining if ovum pick-up at ovulation and tubal transport of both egg and sperm are inhibited by ectopic endometrium.

Publications

1. diZerega, G.S. and Hodgen, G.D.: Prevention of Post-Surgical Tubal Adhesions: Comparative Study of Commonly Used Agents. Am. J. Obstet. Gynecol. 36: 173-178, 1980.

2. diZerega, G.S. and Hodgen, G.D.: Endometriosis: Role of Ovarian Steroids in Initiation, Maintenance, and Suppression. Fert. Steril. 33: 649-654, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00915-01 PR

PERIOD COVERED

January 15, 1980 to August 31, 1980

TITLE OF PROJECT (80 characters or less)

Allogeneic Bone Transplantation In Utero

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	M. Michejda	Special Consultant	PRB	NICHD
Other:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
	J. Bacher	Surgery Unit	VRB	DRS

COOPERATING UNITS (if any)

Surgery Unit VRB, DRS

LAB/BRANCH

Pregnancy Research Branch

SECTION

Section on Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The vitality of bone tissues and their rapid growth in fetuses have been subjects of many investigations. However, bone transplantation in utero has never been accomplished in nonhuman primates. The allogeneic bone transplantation has been performed on twenty rhesus monkeys on 120-135 day of gestation and no allograft rejection was observed. Two techniques were applied. In the first the allograft (bone plug) of fetal humerus was transplanted from fetus (donor) to fetus (recipient). In the second allogeneic paste (small bone particle mixed with a tissue culture medium) was implanted in the same area of humeral midshaft as in the first technique. In both techniques no rejection of the allograft was noted. The fetal-maternal immunologic properties together with the inherent growth potential of the highly vascular fetal bone contribute to rapid healing and normal osteogenesis in the transplanted area. Thus, our results provide a feasibility for a new surgical basis for intrauterine corrective intervention in some congenital bone defects.

PHS-6040

Project Description ZO1 HD 00915-01 PR

Objectives and Methods

The feto-placental allograft and malignant neoplasm represent the only two biological conditions in which antigenic tissue is fully tolerated by an intact immune system. Thus, our objectives are to study the achievement of competent immune surveillence in fetal and neonatal monkeys with the respect to bone transplantation. Fetal bone transplantation will be very useful for early correction of some congenital disorders which result in anomalus skeletal development. Two techniques of intrauterine bone transplantations were performed on partly delivered Macaca mulatta (120-135 days of gestation).

1. The allograft (bone plug) of fetal humerus was transplanted from fetus (donor) to fetus (recipient). The exposed fetal arms were x-rayed following the transplantation procedures. The x-rays of the operated arms were also taken on the first day postpartum as well as at four and eight weeks postnatally. Thus, the radiologic observations of healing processes and normal osteogenesis were continued from the moment of transplantation to the complete bone union in the grafted area.

2. Allogeneic bone paste which consisted of small bone particles mixed with tissue culture media (the same pH as the fetal bone) was implanted in the same area of the fetal humerus as in the first technique. The radiologic observations were carried out in the same fashion as in the "bone plug" technique.

These two successful surgical approaches to the repair of some skeletal defects demonstrated the same immunological phenomena of non-rejection of the allografts and the extensive donor-recipient immunologic tolerance during pregnancy. At this time our findings do not indicate at what point the fetomaternal immunologic properties will disappear and the allograft will be rejected in neonatal or postnatal stage of development. Thus, the next step in our studies will be to estimate the point in the postnatal development when the bone transplants (allografts) will start to be rejected. This will be done by performing the allogeneic transplantations on neonates as well as in four week and eight week old monkeys. Moreover, our two surgical techniques will be applied to intrauterine surgical correction of teretologically induced skeletal defects in monkeys.

Significance: Many congenital disorders result in extensive skeletal defects diagnosed at birth. The magnitude of these defects usually increases in the postnatal stage of bone development. Our study offers the possibility of intrauterine corrective intervention in some congenital bone malformations.

Publications

1. Michejda, M., Hodgen, G.D., Bacher, J.: Allogeneic bone transplantation in utero. (in preparation for publication).

2. Michejda, M., Bacher, J., Hodgen, G.D.: The allograft intramedullary fixation in intrauterine transplantations. (in preparation for publication).

3. Michejda, M., Kuwabara, T., Hodgen, D.G.: Use of allogeneic "bone paste" in intrauterine transplantation. (in preparation for publication).

NICHD ANNUAL REPORT
October 1, 1979 through June 30, 1980
Developmental Pharmacology Branch

SUMMARY

The DEVELOPMENTAL PHARMACOLOGY BRANCH studies the molecular mechanisms underlying genetic differences involved in the stimulation of pharmacologically important enzyme activities by drugs and various environmental contaminants and subsequent metabolism by these enzymes. Why does the same dose of the same drug or other compound given to two different individuals (with the exception of monozygotic twins) evoke different responses? The responses not only include therapeutic effectiveness of a drug, but also include undesirable side effects such as tissue necrosis, cancer, and birth defects. Our studies are conducted with a variety of biological systems, each chosen so as to provide unique experimental access and unequivocal answers to the questions being asked. From results obtained with such experimental systems as inbred strains of mice and established cell culture lines, we can form hypotheses which can be quickly tested in clinical patients. The Branch presently comprises one Section and three Units.

A. The Section on Pharmacogenetics and Molecular Teratology, under the direction of Daniel W. Nebert, M.D., studies the relationship between the etiology of birth defects and the transplacental effects of environmental stimuli (drugs and other foreign chemicals) on such enzyme activities. In certain instances, numerous pharmacogenetic disorders in man cause individuals to react very differently to the same dose of the same drug. The teratogenic, carcinogenic, or toxic effects of certain drugs and other foreign compounds (xenobiotics) may also reflect important genetically mediated differences between individuals. Accordingly, our laboratory has developed experimental model systems for studying drug metabolism in cell culture and in a colony of inbred strains of mice. We have determined that the induction of at least two dozen drug-metabolizing enzyme "activities" is regulated at one or two genetic loci, called the Ah complex for aromatic hydrocarbon responsiveness. The relative "aromatic hydrocarbon responsiveness" among siblings in the uterus or as children results in genetic differences in: polycyclic hydrocarbon-initiated tumors, aplastic anemia, or leukemia; paralysis times when given a certain muscle relaxant; survival times when dosed with several environmental pollutants; hepatic necrosis and cataract formation when given acetaminophen; number of fetal resorptions, stillbirths, and malformations when the pregnant mother is given certain chemicals; and ovarian toxicity and infertility. There now exists considerable evidence for the Ah locus in the human. These genetically mediated dissimilarities in xenobiotic metabolism may therefore attempt to explain in the human why "drug-induced syndromes" occur in one child although the mother has received the same dose of the same drug for two or more pregnancies. We have developed an assay for the Ah receptor, which appears to be the main regulatory gene product for this complex. We have developed antibodies to several of the structural gene products (membrane-bound forms of cytochrome P-450) for use as probes to understand further the genetic regulation. With the aid of these antibodies, we have been able to "size" mRNA associated with the Ah complex, through the use of translation in vitro. Knowing the size, we have now enriched for this mRNA and have several lines of proof for cloned cDNA associated with the Ah complex. With these cloned genes, we should know soon the answers to such questions as genetic linkage, mechanisms of regulation of P-450 induction, and evolutionary phylogeny of this enzyme system (which exists in plasmids, apparently all plants, and apparently all animals).

B. The Unit on Genetic Regulation of Drug-conjugating Enzymes, under the direction of Ida S. Owens, Ph.D., is interested in genetic regulation of basal and inducible glucuronide-conjugating enzyme activities among inbred strains of mice and in cell culture. Detoxification of certain highly lipophilic endogenous substrates and noxious foreign compounds, such as environmental pollutants, requires a multistep process involving phase I and II enzyme systems. With inbred strains of mice, research in this Branch has established that certain monooxygenases (phase I enzymes) are regulated at a few genetic loci and that expression of enzyme induction is dominant or codominant in response to aromatic hydrocarbon treatment. Since certain enzymatic products of the monooxygenase system are often toxic intermediates which can serve as substrates for conjugation (detoxification) via the UDP glucuronosyltransferase system (phase II enzymes), we are eager to determine the extent of coupling between these two enzyme systems in various tissues. We have shown that induction of several hepatic UDP glucuronosyltransferase activities by aromatic hydrocarbons is genetically linked to induction of the monooxygenase system. Furthermore, our studies concerning mutagenicity have shown that induced transferase activity can prohibit the further metabolism and conversion of hydroxylated metabolites of the monooxygenase system to mutagenic products. With bilirubin as substrate, the transferase in certain inbred strains (aromatic hydrocarbon responsive) of mice has two inducible peaks of activity while the transferase in other strains (aromatic hydrocarbon nonresponsive) has one peak of inducible activity. These patterns of inducibility in animal models indicate that in nature (perhaps in humans) more than one form of bilirubin UDP glucuronosyltransferase activity exist. More than one bilirubin transferase activity in humans is also suggested by the different modes of inheritance of defective transferase activity. A consideration of the metabolic pathway for foreign compounds shows that initially monooxygenase inserts a substituent group before one of several other drug-metabolizing enzymes can act on the compound either to toxify or detoxify the compound. Hence, an understanding of the functional relationship (i.e. balance) of each of these enzymes in various tissues might explain target tissue and carcinogenesis, toxicity, and mutagenesis of certain xenobiotics.

C. The Unit on Physical Biology, under the direction of Jack S. Cohen, Ph.D., examines the structure and interactions of biological molecules in detail in solution using several nuclear magnetic resonance spectroscopic methods. An alternating conformation has been shown to characterize the phosphodiester backbone of double-stranded poly(dAdT) in solution from the observation of a bifurcated ^{31}P NMR signal. This result may indicate the presence of sequence-specific local variations in the helical structure of DNA, which may have implications for protein-DNA recognition. The ^{31}P NMR technique has also been applied to measure noninvasively the internal pH of cells. We have carried out the first titration of internal ATP, in chromaffin granules, and have shown that calibration methods for internal pH used until now have been inadequate. Using synthetic phospholipid vesicles we have recently extended this study to divalent metal ion effects on the titration of internal Pi. We have prepared the first singly deuterium-labeled protein, [ε -^{2}H]His 15 hen egg white lysozyme, and have used deuterium NMR to study its mobility and self-association in solution. Due to the particular magnetic properties of ^{2}H, this may be a general method for such measurements. We have used ^{13}C as well as ^{2}H as nondisturbing stable isotope probes to monitor the methionine methyl group directly attached to the heme iron atom in cytochrome c, and are currently studying the conformation and ion-binding properties of this molecule. We have similarly used selective ^{13}C enrichment to study the

binding of synthetic amino-terminal peptides of RNase in the RNase S system. In a recent unique application of this method we have quantitatively followed the peptide exchange process as a function of time and derived dissociation constants. We are now beginning a study of the binding of proteins and drugs to specific sequence double-stranded polynucleotides using the above methods. (All of the above studies provide information on complex biological systems not readily amenable by other techniques).

D. The Unit on Genetic Expression, under the direction of Howard J. Eisen, M.D., is concerned with biochemical and molecular aspects of hormone action during fetal development. Fetal liver is used as a cellular model for analyzing the mechanism of glucocorticoid hormone action. The glucocorticoid receptor is purified by DNA-affinity chromatography, and anti-receptor antibodies are prepared for use in determining the chemical structure and biochemical function of the receptor. The role of glucocorticoids in controlling the development of carbohydrate metabolism in fetal liver is studied in utero and in vitro using a chemically defined organ culture system. The fetal liver organ culture system is also used to study the synthesis of insulin-like growth factors (somatomedins) at various stages of fetal development, and the synthesis of major secretory proteins (α-fetoprotein and albumin).

E. Clinical Research Protocols in this Branch include (i) kinetics of polychlorinated biphenyls concentration in breast milk during the first 6 months of lactation, (ii) attempts to develop a bio-assay for the determination of "breast milk jaundice factor," and (iii) family studies in an assessment of allelic differences at the Ah locus with: idiopathic aplastic anemia, solid tumors, childhood leukemia, and retinitis pigmentosa.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00032-07 DP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Conformations and Interactions of Proteins and Nucleic Acids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jack S. Cohen	Research Chemist	DP NICHD
OTHER:	Abel Schejter	Tel-Aviv University	Israel
	Jan B. Wooten	Staff Fellow	DP NICHD
	Jay L. Zweier	Guest Worker	DP NICHD
	C. L. Chatterjee	Visiting Fellow	LC NIAMDD
	James McGhee	Staff Fellow	LMB NIAMDD

COOPERATING UNITS (if any)

Laboratory of Chemical Physics, NIAMDD (for spectrometer maintenance)

LAB/BRANCH
Developmental Pharmacology Branch

SECTION
Unit on Physical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
32/12	32/12	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To elucidate the conformation and interactions of proteins and nucleic acids in solution by nuclear magnetic resonance (NMR) methods. Particularly to investigate the interactions of proteins and drugs with DNA, alone and in nucleoprotein complexes such as nucleosomes.

Project Description: Z01 HD 00032-07 DP

Objectives: 1. To extend the study of protein functional groups (such as Met, Asp, Thr, etc.) which are not amenable to study by methods other than NMR spectroscopy. 2. To study conformations, transitions and binding properties of biologically active peptides and proteins in solution. 3. To investigate the detailed conformations of DNA and their relationship to genetic function, particularly DNA-protein and drug binding.

Methods Employed: High field NMR spectroscopy (^{1}H, ^{13}C, ^{31}P and ^{2}H) and optical spectroscopies (UV, CD). Synthesis of appropriate substances (peptides, oligonucleotides) including use of isotopic enrichment (^{2}H, ^{13}C).

Major Findings:

A. Conformational Transitions of Cytochrome c by ^{13}C and ^{2}H NMR Methods: The conformational transitions of cytochrome c induced by acid and base have been studied by ^{13}C and ^{2}H NMR methods. The overall protein conformation was monitored by observing the natural abundance (1%) ^{13}C spectrum. The two methionyl methyl groups were also selectively monitored in samples enriched in ^{13}C and ^{2}H, prepared by substitution with the appropriate isotopically labeled methyl iodide.

The overall acid transition of ferricytochrome c occurred with an apparent pK of ca. 3.4, as indicated for example by the collapse on raising pH of the well-resolved Thr Cβ singlet into multiple resonances representing the native protein micro-environments. The ^{13}C resonance of Met 80 was not observed in [ε-^{13}C]Met 65, Met 80 ferricytochrome c down to even pH 1 because of the proximity of this residue to the paramagnetic Fe(III) atom, to which it is bound in the native form. The ^{2}H spectrum of [ε-^{2}H]Met 65, Met 80 ferricytochrome c indicated that Met 80 is exchanging rapidly between Fe(III)-bound and unbound forms as the pH is lowered, since the resonance at -24.7 ppm, corresponding to bound Met 80 in the native form, shifts downfield and broadens below pH 4 (the resonance of Met 65 is unchanged). The novelty of the ^{2}H NMR method is that it is possible to unambiguously monitor the Met 80 residue in both Fe-bound and unbound conformations simultaneously.

The alkaline transition of ferricytochrome c was followed by measuring the relative areas of the ^{2}H NMR peaks corresponding to bound and unbound [ε-^{2}H]Met 80, and from the reappearance of the ^{13}C signal of [ε-^{13}C]Met 80 as the pH was raised. Titration curves yielded pK values varying from 7.6 to 9.1 and Hill coefficients (n) from 0.75 to 1.0 for different samples, and these values were confirmed by UV monitoring of the 695 nm band arising from the Met 80 S-Fe bond. It appears that these variations arise from conformationally altered samples resulting from extended alkaline treatment and/or extensive chromatographic purification.

B. Characterization of Alternating DNA Conformations: Medium length (50-200 bp alternating purine-pyrimidine DNAs were prepared by sonication of synthetic polymers at low temperature. The products were sufficiently small for high resolution ^{31}P NMR studies. While two of the sequences, d(GC)·d(GC)

and d(AU)·d(AU), gave singlet ^{31}P resonances, three other sequences, d(AT)·d(AT), d(A5BrU)·d(A5BrU) and d(IC)·d(IC), exhibited two resolved signals of equal area. This indicates the existence of alternating phosphodiester backbone conformations for these three B-DNAs in solution. Controls of block co-polymers, which were also prepared by sonication, showed only singlet ^{31}P resonances. Of the alternating sequence DNAs, only sonicated poly(dGdC)·poly(dGdC) exhibited a conformational transition to a high salt (>2.5 M) form. This exhibits two well-resolved ^{31}P resonances of equal area which also indicates an alternating backbone structure, which is presumed to be a Z-DNA. These results indicate a general response of the DNA backbone conformation to alternating purine-pyrimidine base sequences, but with a degree of specificity which might have functional genetic significance.

C. Phosphate Binding of DNA in Nucleosomes: Nucleosomes are the primary structural elements of the gene. Nucleosome core particles contain DNA (145 bp) wrapped around a histone octomer core. The DNA exhibits many periodicities to cleavage by various nucleosomes. The question arises if this results from a degree of inaccessibility of the DNA bound to the histones. To test this ^{31}P NMR spectra of nucleosome core particles were monitored upon the stepwise addition of Mn^{2+}. Since this ion is paramagnetic the ^{31}P signal broadens as Mn^{2+} binds closely to the phosphodiester groups in DNA. Two interesting facts were found, (a) that nearly all (ca. 95%) of the phosphates are accessible to Mn^{2+}, and only ca. 5% (error ca. $\pm$ 5%) are relatively inaccessible, (b) that the accessibility of phosphates of DNA in nucleosomes is quantitatively the same as that of naked DNA. The conclusions are that the DNA in nucleosomes is highly accessible to solvent, and that the periodicities observed in nuclease cleavage must arise from steric factors preventing accessibility of the enzyme to the DNA.

D. Conformation of hCG Peptides: It has been found that carboxyl terminal peptides of human chorionic gonadotropin (hCG) exhibit biological activity and full binding to specific-hCG antibodies if they are >15 amino acid in length. The conformation in solution of synthetic peptides of various lengths is being investigated by the ^{13}C NMR method. ^{13}C NMR spectra should reveal by comparison with spectra simulated on the basis of a random coil structure if some of these peptides exhibit any specific folded structure, which might correlate with their biological activity.

Significance to Biomedical Research and the Program of the Institute: The results of this work are of fundamental significance in understanding the actual detailed structure of DNA and its relationship to function. Continuing NMR studies of protein conformations and of protein-DNA complexes and their interactions with drugs in solution should reveal further insights of important physico-chemical processes in developmental molecular biology.

Proposed Course: (1) Extensive studies are planned to relate the conformational variants of DNA now discovered to actual functional properties of DNA; particularly DNA-protein and DNA-drug interactions will be investigated. For this work medium length specific-sequence DNAs will be required, both synthetic and of natural occurrence. Also, the role of acetylation and phosphorylation in histone function will be investigated if feasible. (2) Attempts will be made to extend the use of CD_3-labelled Met cytochrome c to

its interaction with cytochrome oxidase, possibly in a membrane-like environment. (3) Studies of peptide and protein conformation will be continued, with hCG and possibly catalase. Proton NMR studies of larger proteins will be re-initiated when our high field (500 MHz) spectrometer is delivered in the coming year.

Publications:

1. Shindo, H., Simpson, R., and Cohen, J.S.: An alternating conformation characterizes the phosphodiester backbone of poly(dAdT) in solution. J. Biol. Chem. 254: 8125-8128, 1979.

2. Shindo, H., McGhee, J., and Cohen, J.S.: ^{31}P NMR studies of DNA in nucleosome core particles. Biopolymers 19: 523-537, 1980.

3. Shindo, H., Matsuura, S., and Cohen, J.S.: Conformation of ribonuclease S-protein. Experientia 35: 1284-1285, 1979.

4. Niu, C., Shindo, H., Matsuura, S., and Cohen, J.S.: Direct observation of peptide exchange by stable isotope enrichment. J. Biol. Chem. 255: 2036-2038, 1980.

5. Cohen, J.S., Niu, C., Matsuura, S., and Shindo, H.: Conformation, mechanism and peptide-exchange of ^{13}C-enriched ribonuclease-S by ^{13}C NMR spectroscopy. In: Frontiers in Protein Chemistry. Elsevier, 1980, in press.

6. Wooten, J.B., Cohen, J.S., and Schejter, A.: Conformational transitions of ferricytochrome c studied by ^{13}C and ^{2}H NMR spectroscopy. In: Interaction Between Iron and Proteins in Oxygen and Electron Transport. Elsevier, 1980, in press.

7. Cohen, J.S.: DNA; is the backbone boring? Trends in Biochem. Sci. 5: 58-60, 1980.

8. Wooten, J.B., and Cohen, J.S.: Characterization of alternating DNA conformations. Fed. Proc. 39: 1879, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00033-07 DP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Application of Stable Isotopes to Biochemical Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jack S. Cohen Research Chemist DP NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental Pharmacology Branch

SECTION
Unit on Physical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/12	3/12	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To develop the use of stable isotopes (^{13}C, ^{15}N, ^{2}H) as non-disturbing probes in biochemical systems with the application of appropriate NMR techniques. In order to accomplish this objective the synthesis of molecules containing these isotopes enriched at selected positions is undertaken.

PHS-6040

Project Description: Z01 HD 00033-07 DP

Objectives: To develop probes of molecular structure and function by utilizing the combination of stable isotopic substitution (^{13}C, ^{15}N, ^{2}H, ^{77}Se) with NMR observation of the isotopic probe.

Methods Employed: Nuclear magnetic resonance observation of the appropriate nuclei.

Major Findings: Methods developed in this project have been applied to problems in other projects.

Significance to Biomedical Research and the Program of the Institute: (1) Interactions and conformations of macromolecules in solution can be studied by relatively few techniques. It is important to develop new techniques towards this end in order to obtain fundamental information about macromolecular function. (2) Use of stable isotopes in metabolic studies could be of great significance to neonatal systems.

Proposed Course: Discontinued since NMR methods successfully developed now transferred to applications in other projects.

Publications:

1. Niu, C., Bertrand, R., Shindo, H., and Cohen, J.S.: Cross-peptide bond ^{13}C-^{15}N coupling constants by ^{13}C and J cross-polarization ^{15}N NMR. J. Biochem. Biophys. Methods 1: 135-144, 1979.

2. Wooten, J., and Cohen, J.S.: Protein mobility and self-association by deuterium NMR. Biochemistry 18: 4188-4191, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00136-12 DP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

PHARMACOGENETICS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. W. Nebert Chief, DPB & Head, Section on Pharmacogenetics and Molecular Teratology - NICHD

OTHER:

L.S. Andrews	Guest Worker	DP NICHD
S.W. Bigelow	Chemist	DP NICHD
W.F. Dodge	Guest Worker	DP NICHD
M.G. MacDonald-Ginzburg	Guest Worker	DP NICHD
N.M. Gordon	Chemist	DP NICHD
L.M. Hjelmeland	Senior Staff Fellow	DP NICHD
B.M. Kisliuk	Junior Fellow	DP NICHD
M.A. Lang	Visiting Fellow	DP NICHD
C. Legraverend	Visiting Fellow	DP NICHD
M. Negishi	Visiting Scientist	DP NICHD
L. Soyka	Guest Worker	DP NICHD
H.W. Tate	Biological Aid	DP NICHD

COOPERATING UNITS (if any)

SEE ATTACHED

LAB/BRANCH
Developmental Pharmacology Branch

SECTION
Section on Pharmacogenetics and Molecular Teratology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
89/12	63/12	26/12

SUMMARY OF WORK (200 words or less - underline keywords)

In certain instances, human pharmacogenetic disorders cause individuals to react very differently to the same dose of the same drug. The teratogenic, carcinogenic, or toxic effects of certain drugs and other foreign compounds (xenobiotics) also may reflect important genetically mediated differences between individuals. Accordingly, our laboratory has developed experimental model systems for studying drug metabolism in cell culture and among inbred strains of mice. We have found that the induction of more than two dozen drug-metabolizing enzyme activities is regulated by a genetic system called the Ah complex. We have characterized the major regulatory gene product, a cytosolic receptor that binds avidly to certain inducers of P-450; the inducer-receptor complex undergoes a temperature-dependent translocation into the nucleus. Among the structural gene products are multiple forms of P-450--a minimum of 12 induced by way of the Ah receptor. Cloned cDNA associated with mouse liver P_1-450 has been isolated and characterized. This entire genetic complex has been a useful probe in demonstrating differences in individual risk for various drug-induced birth defects, other forms of drug toxicity, and environmentally-caused malignancies.

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Project Description:

Objectives: Membrane-bound monooxygenases are important in the metabolism of drugs, carcinogens, and insecticides, as well as many endogenous lipophilic substrates such as steroids, fatty acids, cholesterol, indoles, biogenic amines, and thyroxine. We have found that the "induction," or stimulation, of a group of drug-metabolizing enzyme activities in response to aromatic hydrocarbon treatment is regulated by only a few genetic loci in the mouse. We have also employed cell culture techniques for studying the mechanism of microsomal monooxygenase induction in an experimental system which is more rigidly controlled than similar studies in the intact animal. Understanding the mechanism by which drug-metabolizing enzymes are induced may elucidate important concepts in the fields of drug metabolism, developmental pharmacology, steroid biochemistry, chemical carcinogenesis, teratology, entomology, and limnology. The main objectives of this project are (1) to exploit genetic differences in drug metabolism between various inbred strains of mice in order to understand better the potential importance of pharmacogenetics; (2) to use tissue culture techniques in answering questions which would be difficult or impossible with studies in the intact animal; (3) to use recombinant DNA technology to understand better the genetic linkage and evolutionary development of these drug-metabolizing enzymes; (4) to combine the genetic differences with the tissue culture and recombinant DNA techniques in order to elucidate certain mechanisms related to mammalian gene regulation; and (5) to apply certain of these techniques to human tissues so that extrapolations can be made from rigidly controlled experimental systems to human pharmacogenetic differences.

Methods Employed: The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations of product formation or substrate disappearance, (3) radioisotopic measurements using tritium and carbon-14, (4) cell culture techniques, (5) differential centrifugation, (6) phase microscopy, (7) polyacrylamide gel electrophoresis, (8) isoelectric-focusing electrophoresis, (9) column- and thin-layer chromatography, (10) magnetic resonance techniques, (11) high pressure liquid chromatography, (12) gas chromatography, (13) gas chromatography-mass spectrometry, (14) *in vitro* translation of mRNA, (15) peptide mapping *via* partial proteolysis, and (16) recombinant DNA technology.

Major Findings: A. Teratology. (1) Benzo[a]pyrene, at doses between 50 and 300 mg per kg body weight given at Day 7 or 10 of gestation, causes *in utero* toxicity and teratogenicity more so in genetically "responsive" C57BL/6 than in "nonresponsive" AKR inbred mice. With the use of AKR x (C57BL/6)(AKR) and (C57BL/6)(AKR) x AKR backcrosses, it was shown that allelic differences at the *Ah* locus in the fetus can be correlated with dysmorphogenesis. Of particular interest in our study is the fact that the mother and the father both must be of a particular genotype before differences in teratogenesis among fetuses (due to *their* genotype) will be expressed. These data might provide an example in attempting to explain clinically why only one child is affected with an apparent "drug-induced syndrome" although the mother has taken the same dose of the particular drug during each of numerous pregnancies.

(2) Mouse embryos explanted at 7½ or 8½ days of gestation were cultured in medium containing benzo[a]pyrene and supplemented with 5-bromodeoxyuridine to allow detection of sister chromatid exchanges. A high frequency of sister chromatid exchange was induced by benzo[a]pyrene in embryos from three Ah-responsive inbred strains--BALB/cDub, C3H/AnfCum, and C57BL/6N; there was little or no increase in two Ah-nonresponsive inbred strains, AKR/J and DBA/2J. Benzo[a]pyrene also induced sister chromatid exchanges in the Ah-responsive recombinant inbred line B6NXAKN-12 but not in the Ah-nonresponsive recombinant inbred line B6NXAKN-3. These data provide direct evidence that genetically responsive mouse embryos (early post-implantation stage) possess the subcellular processes necessary for induction of enzymes that metabolize benzo[a]pyrene to its chemically active form(s). Both the Ah regulatory gene product (a cytosolic receptor) and the structural gene product (inducible cytochrome P_1-450) therefore appear to be functional from an early embryonic age. (3) Plasma phenytoin elimination rates were examined among 12 inbred strains of mice. Two populations are identified--the "fast metabolizers" having almost exactly twice as rapid an elimination rate as the "slow metabolizers." The phenytoin elimination rate in the (C57BL/6J)(DBA/2J)F_1 heterozygote is expressed as an additive trait. A good correlation exists between phenytoin elimination rates in vivo and phenytoin metabolism by liver microsomes in vitro, as determined by a newly-described assay using high-performance liquid chromatography. The cytochrome P-450-mediated monooxygenase metabolism of phenytoin is not associated with the Ah locus or with coat color among progeny of the (C57BL/6N)(DBA/2N)F_1 x DBA/2N backcross. The marked sensitivity of inbred mouse strains CL/FR and A/J and the marked resistance of STAR/N, Swiss-Webster, and C57BL/6 to phenytoin-induced cleft lip and/or palate cannot be explained by genetic differences in phenytoin elimination rates or liver microsomal metabolism in vitro, as measured by the methods used in this study.

B. Toxicology. (1) Following induction of total body cytochrome P_1-450 by 3-methylcholanthrene, there is a strong correlation between the Ah^b allele and cataract formation caused by large doses of intraperitoneal acetaminophen (1,000 mg/kg body weight) among 4 inbred mouse strains genetically responsive at the Ah locus, 5 inbred strains genetically nonresponsive, and progeny of the (C57BL/6N)(DBA/2N)F_1 x DBA/2N backcross. There is no apparent depletion of reduced glutathione levels in the lens of either strain. The mechanism of ocular toxicity without glutathione depletion may differ from that of hepatic necrosis, which occurs only when glutathione is depleted. Daily oral doses of acetaminophen or naphthalene (60-120 mg/kg/day) cause cataract formation in C57BL/6N concomitantly treated with 3-methylcholanthrene or β-naphthoflavone; no DBA/2N mice develop cataracts under these regimens. Some of these cataracts persisted in otherwise healthy C57BL/6N until they were killed for examination 6 or 7 months later. Histological signs of chronic toxicity were seen in the anterior lens cortex, charoid, ciliary body, and iris. Because drug-induced cataracts look clinically very similar to senile cataracts, these results may be important to patients receiving either a single large overdose of acetaminophen or high doses over a long period of time. (2) Allelic differences at the Ah locus are reflected in the large differences in inducibility of cytochrome P_1-450 and benzo[a]pyrene metabolism in numerous tissues when the mice receive the chemical daily in their diet. The result

is lethality associated with toxic chemical depression of the bone marrow in the genetically nonresponsive Ah^d/Ah^d individual and little or no toxicity in the genetically responsive Ah^b/Ah^d heterozygote. The differences in toxicity are shown to be caused by a marked destruction of myeloid and platelet precursors and less destruction to the erythroid precursors. This experimental model system offers to the hematologist and clinical pharmacologist a means to study genetic differences in toxic chemical depression of the bone marrow, as well as a potential model for aplastic anemia and leukemia explainable on a single-gene basis. (3) The genetically responsive individual who is at increased risk for cancer caused by subcutaneous or topical or intratracheal polycyclic hydrocarbons is at decreased risk for toxicity of the bone marrow and leukemia caused by oral benzo[a]pyrene, (when compared with the genetically nonresponsive individual receiving the same dose of the same xenobiotic). In other words, tissue sites in direct contact with the carcinogen develop cancer in responsive animals because of induced P_1-450; tissues in distant sites of the body may develop malignancy in nonresponsive animals because more carcinogen reaches that tissue due to decreased P_1-450 induction all over the body and therefore decreased detoxification. Not only the dose but the route of administration is therefore very important in the interpretation of data from tumorigenesis or toxicity experiments involving P_1-450 inducers such as polycyclic hydrocarbons.

C. Cancer Research. (1) Allelic differences at the Ah locus are shown to exist in the mouse brain. This finding probably explains inbred mouse strain differences in polycyclic hydrocarbon tumorigenesis of the brain described more than 35 years ago and may be important in understanding the etiology of genetic differences in certain human intracranial neoplasms. (2) C57BL/6, BALB/c, and C3Hf/He are three highly inbred mouse strains that are all genetically responsive with regard to the Ah locus but exhibit important differences in sensitivity to epidermal carcinoma when benzo[a]pyrene is applied topically three times weekly. Epidermal benzo[a]pyrene-initiated carcinoma is, therefore, closely correlated to the relative amounts of induced benzo[a]pyrene metabolism in the target cell. These results are in contrast to previous data in which C3H mice were greater than five times more sensitive than C57BL/6 mice to fibrosarcomas initiated by subcutaneously administered benzo[a]pyrene. Tumorigenesis data based on the subcutaneous injection of chemicals in mice derived from C3H stock should, therefore, be viewed with caution.

D. Mutation Research. (1) Pyrolysis products of proteins and amino acids are highly mutagenic, but metabolism of these chemicals by rat liver subcellular fractions is known to be required for production of the mutagenic intermediates. We examined the mutagenesis of seven purified pyrolysis products from tryptophan, lysine, glutamic acid, and soybean globulin with Salmonella typhimurium strain TA98 in the presence of liver fractions from genetically responsive C57BL/6N and Ah^b/Ah^d or nonresponsive DBA/2N and Ah^d/Ah^d mice that had been pretreated in vivo with benzo[a]pyrene. The data indicate that the pyrolysis products tested, although not effective as inducers of cytochrome P_1-450, are most mutagenic when metabolized by P_1-450. Potent P_1-450 inducers--present in pyrolysates during the combustion process--might be present in quantities insufficient to initiate mutagenesis or carcinogenesis but might

have a synergistic action, or act as "comutagens" or "cocarcinogens," with the N-containing heterocyclic pyrolysis products. (2) Hexane extracts of four commercial preparations of coal tar shampoos were studied for their mutagenic properties in the Salmonella/liver test system in vitro. Three of the four shampoos were highly mutagenic, whereas the fourth was not--under our experimental conditions. By high-performance liquid chromatographic, gas-liquid chromatographic, and gas-liquid chromatography-mass spectrometric analyses, more than 35 distinct fractions could be resolved; seven polycyclic aromatic chemicals believed to be present in coal tar were tentatively assigned as the major component of some of these fractions. The shampoo extract that was most mutagenic had a greater number of distinct fractions and contained approximately 50 times more benzo[a]pyrene, compared with the one shampoo extract that was not mutagenic under our experimental conditions.

E. Drug Metabolism. (1) Ellipticine (5,11-dimethyl-[6H]-pyrido[4,3b]carbazole) binds with an affinity greater than most other compounds known to interact with P-450. Control and 3-methylcholanthrene-induced aryl hydrocarbon (benzo[a]pyrene) hydroxylase and acetanilide 4-hydroxylase and control and phenobarbital-induced ethylmorphine N-demethylase activities are all markedly inhibited by ellipticine to about the same extent. Ellipticine and other Type II compounds (metyrapone, octylamine-1, pyridine and aniline) preferentially inhibit NADPH-cytochrome P-450 reductase activity, while affecting NADPH-cytochrome c reductase activity very little. The data indicate that ellipticine may be very useful in attempting to understand the mechanism by which electrons are transferred from the reductase to the cytochrome(s) P-450. (2) The binding of reactive benzo[a]pyrene metabolites to deproteinized DNA in vitro can be drastically changed, both quantitatively and qualitatively, in vitro by changes in the substrate concentration or the substrate/P-450 ratio, and in the intact animal by starvation or substitution of a "purified protein test diet" for the regular laboratory chow. The data demonstrate the importance of dietary contaminants or nutrition during benzo[a]pyrene tumorigenesis. "Untreated" genetically responsive mice are shown to have greater "control" levels of benzo[a]pyrene metabolism in their various tissues than genetically nonresponsive mice simply on the basis that responsive mice have a lower threshold for "responsiveness" to exogenous inducers inhaled and/or ingested in their crude diet, i.e., responsive "control" animals already have partially induced enzymes. (3) The subcellular distribution of aryl hydrocarbon hydroxylase, NADPH-cytochrome c reductase, NADH-cytochrome c reductase, and NADH-cytochrome b_5 reductase activities in mouse liver was studied using the biochemical membrane markers microsomal glucose-6-phosphatase, mitochondrial cytochrome c oxidase, and plasma membrane 5'-nucleotidase The nuclear fraction contains less than 1% of the total cellular activities of the hydroxylase and all three reductases. All detectable basal aryl hydrocarbon hydroxylase activity in the nuclear fraction of control C57BL/6N and DBA/2N and 3-methylcholanthrene-treated DBA/2N mice and all detectable NADPH-cytochrome c, NADH-cytochrome c, and NADH-cytochrome b_5 reductase activities in the nuclear fraction of control and 3-methylcholanthrene-treated C57BL/6N and DBA/2N mice can be completely accounted for by the degree of microsomal fragment contamination (as assessed by the microsomal marker glucose-6-phosphatase). However, there is more induced aryl hydrocarbon hydroxylase activity in the nuclear fraction of 3-methylcholanthrene-treated C57BL/6N mice than can be accounted for by the degree of microsomal membrane contribution.

F. Molecular Genetics. (1) All organisms possess a number of genetically regulated mechanisms in order to cope with rapid adverse changes in the environment. The two systems which appear to respond to a seemingly endless array of chemical specificities are the immune response and the induction of drug-metabolizing enzymes. DNA insertion sequences, plasmid "transposons," maize "controlling elements," gene duplication, intervening sequences, and high-frequency intergenic recombination are all possible methods by which organisms can "adapt" quickly to a new selective pressure. If the regulation of P-450 induction resembles in any way the other methods by which pro- and eukaryotes cope genetically with numerous forms of environmental adversity, therefore, it is very likely that mammalian tissues contain hundreds, if not thousands, of inducible forms of P-450. (2) We isolated two inducible forms of partially separated P-450 from C57BL/6N treated with 3-methylcholanthrene, and an antibody to each of these forms was developed. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a molecular weight of about 55,000 is estimated for both partially separated hemoproteins. Limited proteolysis reveals differences in the peptide composition of these two cytochromes. P_1-450 is more closely associated with polycyclic aromatic compound-inducible aryl hydrocarbon hydroxylase activity and NADPH oxidation in the presence of benzo[a]pyrene, whereas P-448 is more closely associated with polycyclic aromatic compound-inducible acetanilide 4-hydroxylase activity and NADPH oxidation in the presence of acetanilide. The data provide direct biochemical evidence that two structural gene products controlled by the Ah locus represent distinctly different forms of P-450. (3) Radioactive phosphorus is not incorporated into either hemoprotein in vivo. Labeled glucosamines are incorporated into P-448, but negligible amounts are incorporated into P_1-450 in vivo. We conclude that P_1-450 probably does not exist in the "pure" control untreated animal, whereas P-448 (or another protein cross-reacting with anti-P-448) exists in control microsomes. (4) A close correlation between induced ornithine decarboxylase and aryl hydrocarbon hydroxylase activities was observed when the inbred C57BL/6N and DBA/2N mouse strains were treated intraperitoneally with β-naphthoflavone, phenobarbital, or a low or a high dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Hepatic and renal ornithine decarboxylase induction by β-naphthoflavone or 3-methylcholanthrene was strictly associated with the Ah^b allele, when genetically responsive Ah^b/Ah^d and genetically nonresponsive Ah^d/Ah^d progeny of the (C57BL/6N)(DBA/2N)F_1 x DBA/2N backcross were compared. Ornithine decarboxylase induction may thus represent an early event during the sequence of subcellular changes between the time that the inducer first enters the cell and the time when induced drug-metabolizing enzyme activities become maximal.

G. Receptor Studies. (1) The major regulatory gene product of the murine Ah locus is a cytosolic receptor. Sucrose density gradient analysis following dextran-charcoal treatment is shown to be far more reliable in characterizing this receptor than DEAE-cellulose column chromatography or previously published procedures involving dextran-charcoal adsorption. Sucrose density gradients clearly separate a class of high affinity, low capacity binding sites from nonsaturable binding sites for 2,3,7,8-[1,6-^{3}H]tetrachlorodibenzo-p-dioxin. With this assay we show that the hepatic cytosolic receptor: (a) binds most specifically to foreign polycyclic aromatic compounds that are effective inducers of cytochrome P_1-450; (b) is saturable; (c) possesses high affinity,

low capacity binding sites; (d) appears to be composed principally of protein; (e) is highly thermolabile, more so in the absence of inducer; (f) does not appear to increase during in vivo treatment with inducers of P_1-450; (g) is present in five responsive inbred mouse strains examined, plus the Sprague-Dawley rat; (h) is not detectable in five nonresponsive inbred strains examined; and (i) is present in the Ah^b/Ah^d heterozygote, but not detectable in the Ah^d/Ah^d homozygote from the (C57BL/6N)(DBA/2N)F_1 x DBA/2N backcross. The apparent mean number of sites detected by saturation analysis in C57BL/6N mice corresponds to about 5,500/hepatic cell (60 fmol/mg of cytosolic protein). The apparent K_d for 2,3,7,8-[1,6-^{3}H]tetrachlorodibenzo-p-dioxin binding is estimated to be approximately 0.7 nm. (2) The mean number of cytosolic high affinity binding sites decreases more than 75% during the first 6 hours following β-naphthoflavone treatment in vivo. 2,3,7,8-[1,6-^{3}H]Tetrachlorodibenzo-p-dioxin treatment in vivo of C57BL/6N or Ah^b/Ah^d mice for 2 to 18 hours results in the appearance of a hepatic nuclear inducer-protein complex that sediments at 5.7 S on sucrose density gradients of high ionic strength following dextran-charcoal treatment. No such peak occurs in similarly treated DBA/2N or Ah^d/Ah^d mice. The data suggest that the cytosolic receptor, and its translocation with specifically bound inducer into the nucleus, are essential during the sequence of cytochrome P_1-450 induction and other events controlled by the Ah locus. (3) Hepatic receptor levels, β-naphthoflavone-inducible and control aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase activities and total P-450 content were studied in the Sprague-Dawley rat, C57BL/6N mouse, New Zealand White rabbit and Sigmoden hispedis (cotton rat) as a function of age. The developmental data indicate that: (a) the form of P-450 responsible for induced aryl hydrocarbon hydroxylase activity is not the same as that for induced acetanilide 4-hydroxylase, and neither one is the same as induced cytochrome P-448; (b) the presence of the TCDD-specific cytosolic receptor per se (detected as a distinct high-specificity saturable peak on sucrose density gradients) does not guarantee the expression of inducible aryl hydrocarbon hydroxylase or acetanilide 4-hydroxylase activity; (c) although interesting developmental differences exist among all four species examined, the TCDD-specific receptor is maximal between the neonatal and weanling period, is considerably decreased in the adult, and is suppressed even more during the latter half of pregnancy; and (d) in general, the times at which the cytosolic receptor is highest or lowest parallels quite closely the well-known increases in inducible drug-metabolizing enzymes that have been commonly observed in the rat, mouse and other laboratory animals.

Significance to Biomedical Research and the Program of the Institute: We have demonstrated that allelic differences at a genetic locus controlling concentration of a receptor in the mouse is associated with: (1) increased susceptibility to 3-methylcholanthrene-initiated tumors; (2) decreased zoxazolamine paralysis time; (3) increased susceptibility to 7,12-dimethylbenz[a]anthracene-produced skin inflammation; (4) increased susceptibility to acetaminophen-induced hepatic necrosis and cataract formation; (5) shortened survival time, or (conversely) apparent protection, when large doses of polycyclic hydrocarbons, lindane, or polychlorinated biphenyls are administered intraperitoneally or orally; (6) apparent protection against aplastic anemia and leukemia caused by oral benzo[a]pyrene; (7) increased susceptibility to stillborns, fetal resorptions, and malformations caused by 3-methylcholanthrene or 7,12-

dimethylbenz[a]anthracene given to the pregnant mother; (8) increased ovarian toxicity (loss of primary oocytes) following polycyclic hydrocarbon treatment; and (9) increased infertility in the female. An increased incidence of lung cancer in man has been correlated with living in highly polluted urban areas. It is therefore possible that differences--caused by a very small number of genes--in man are related to increased susceptibility to cancers, birth defects, tissue necrosis, drug metabolism, bone marrow disorders, and decreased life span caused by exposure to foreign compounds.

These genetically mediated dissimilarities in xenobiotic metabolism have been shown to be a useful probe in demonstrating differences in the rates of formation of certain drug-produced birth defects. Consequently, studies involving the development and extent of induction of mixed-function oxygenases in fetal tissues and in the placenta during various periods of gestation, are relevant to the program of this Institute in the following areas: (1) possible effects of environmental pollutants (i.e., polycyclic hydrocarbons and insecticides) and various drugs on normal fetal growth and development and on the health of the pregnant woman, (2) mechanisms of "pharmacological immaturity" of the premature and newborn, (3) mechanisms of disease of the fetus and newborn concerning oxygenative and conjugative metabolism of normal body substrates such as steroids and bilirubin, and (4) possible therapeutic and diagnostic procedures for aiding and predicting pharmacogenetic defects in the fetus and neonate.

Proposed Course of Project: (1) Delineate further differences in the mechanism(s) of monooxygenase induction by polycyclic aromatic compounds, phenobarbital, and numerous other drugs and environmental pollutants; (2) characterize specific and nonspecific binding of these various inducers to receptors and other subcellular sites; (3) determine differences in metabolite formation, binding of reactive metabolic intermediates to DNA, and biophysical characteristics of the enzyme active-site between control and drug-treated animals or cells in culture; (4) compare these genetic differences with contemporary models of mammalian gene expression and regulation; (5) explore evolutionary development and phylogenetic differences among genes associated with the metabolism of drugs and other environmental pollutants; (6) search for drug metabolism parameters in cultured human lymphocytes or fibroblasts or in various excreta that might aid in predicting a patient's response to a drug or class of drugs; and (7) extrapolate mouse data to clinical diseases such as drug-induced birth defects, toxicity, and environmental carcinogenesis and mutagenesis.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00137-06 DP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

GENETIC REGULATION OF DRUG-CONJUGATING ENZYMES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Developmental Pharmacology Branch

SECTION
Unit on Drug-Conjugating Enzymology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
24/12	24/12	0/12

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Studies concerning the detoxification of noxious, lipophilic foreign and endogenous compounds through oxidation by monooxygenase activity and/or through conjugation by genetically regulated UDP glucuronosyltransferase activity have continued primarily with 1-hydroxy-, 3-hydroxy-, 9-hydroxy-phenols, and bilirubin. Further studies in which analyses for induction of other types of bilirubin conjugating activities were made indicate that a UDP glucuronosyltransferase activity is responsible for genetically regulated induction of bilirubin conjugating activity. Purification of UDP glucuronosyl-transferase activity is markedly improved by using a phosphate buffer system including 20% glycerol with hydrophobic and hydroxyapatite columns which enhance the removal of cytochrome P-450 which is present in much higher levels in microsomes than is the UDP glucuronosyltransferase enzyme(s). Preliminary studies with the antiseizure drug valproic acid indicate that immature mice clear the drug from the blood more slowly than mature animals and develop complete loss of hair from the body after one week of daily injections at a dose consistent with a human dose.

Project Description:

Objectives: The removal of many foreign lipophilic compounds from the body requires both hydroxylation by monooxygenase activity and subsequent conjugation by sulfotransferase(s) or UDP glucuronosyltransferase(s). With inbred strains of mice and offspring from the appropriate backcrosses using two prototypic strains, research in this Branch has established that induction of several monooxygenase activities by aromatic hydrocarbon compounds is genetically regulated by a single gene designated the Ah locus. More recently, our studies have shown that induction of 4-methylumbelliferone-UDP glucuronosyltransferase activity is also genetically mediated and is linked to the Ah locus. The genetic linkage of these two functionally related membrane bound enzymes does not occur through the common intervening hydroxylated product of the monooxygenase activity which is also substrate for UDP glucuronosyltransferase activity. These observations are further substantiated in cell culture studies with benzo[a]pyrene metabolites which are generally less potent inducers than the parent compound. To explore further the nature of the coordinate monooxygenase and UDP glucuronosyltransferase activities, the genetic regulation for induction of bilirubin transferase activity was studied in these prototype mice and was found to be genetically linked to the induction of monooxygenase. The endogenously-derived bilirubin does not require monooxygenase. Since monooxygenases are important in the metabolism of drugs, carcinogens, and insecticides as well as endogenous compounds as fatty acids and cholesterol, it is of interest to investigate the extent to which monooxygenase is coordinated with UDP glucuronosyltransferase activity during the metabolism of a known potential carcinogen and other compounds. The carcinogenicity of benzo[a]pyrene, at least, appears to be associated with oxygenation whereas detoxification appears to be associated with conjugation of oxygenated benzo[a]pyrene. The primary objectives of this project are (1) to delineate further the genetically mediated induction of UDP glucuronosyltransferase (using substrates which are products of the monooxygenase enzyme) in an attempt to determine substrate specificities of induced transferase(s) for various phenols of benzo[a]pyrene; (2) to determine whether induced UDP glucuronosyltransferase, as assayed in vitro, reflects the increased in vivo conjugation of certain endogenous and exogenous substrates; (3) to exploit genetic differences in the conjugation of critical substrates, as bilirubin; (4) to study the functional interrelationship between UDP glucuronosyltransferase and various P-450-mediated monooxygenases in the detoxification of various drugs in vivo in genetically different mouse strains; (5) to purify a genetically regulated and a nongenetically regulated UDP glucuronosyltransferase and compare properties of these enzymes; (6) to use the bacterial mutagenesis system developed by Ames to examine the effect of UDP glucuronosyltransferase on the mutagenicity of certain compounds; (7) to attempt to use tissue culture techniques to study the regulation of mammalian UDP glucuronosyltransferase activity and the interdependence in regulation of the transferase and the monooxygenase at the molecular level; and (8) to apply tissue culture results and an in vitro bilirubin conjugation assay for the purposes of establishing a bioassay for "breast milk jaundice factor."

Methods Employed: The principal methods to be employed are: (1) enzyme assays using spectrophotometric, spectrophotofluorometric, radiometric determinations of product or disappearance of substrate, (2) in vivo radio-

metric measurements, (3) regular cell culture and hybridoma cell culture techniques, (4) differential centrifugation, (5) phase microscopy, (6) high-pressure liquid chromatography, (7) column chromatography, (8) variable temperature regulators, (9) gel electrophoresis, (10) antibody production in rabbit, and (11) immunoprecipitation of UDP glucuronosyltransferase.

Major Findings: Hepatic microsomes from aromatic hydrocarbon-treated C57BL/6N mice further oxidize [^{3}H]1-hydroxybenzo[a]pyrene to highly reactive metabolites which bind to DNA and generate the same types of DNA adducts as seen with [^{3}H]benzo[a]pyrene as the starting substrate for monooxygenase activity. Microsomal metabolism of [^{3}H]1-hydroxybenzo[a]pyrene appears to generate a higher ratio of the more water soluble adducts eluting from a LH-20 column with methanol and water compared to metabolism by S-9 cell fractions where the cytochrome P-450 content is much lower than in microsomes. The results suggest that certain phenolic benzo[a]pyrene metabolites are as reactive after recycling through the microsomal monooxygenase system as metabolites of the native benzo[a]pyrene compound. If 1-hydroxybenzo[a]pyrene is metabolized in the presence of the substrate UDP-glucuronic acid for UDP glucuronosyltransferase the production of [^{3}H]1-hydroxybenzo[a]pyrene metabolite-DNA adducts are reduced, suggesting that glucuronidation prevents further metabolism of the phenol.

Studies with the endogenous substrate, bilirubin, established that at least three different forms of inducible bilirubin UDP glucuronosyltransferase(s) exist in mice. In the responsive-prototype C57BL/6N mouse, aromatic hydrocarbons treatment induces two peaks of bilirubin transferase activities with maxima at 6 and 10 days after a single injection. In the nonresponsive-prototype DBA/2N mouse hydrocarbon treatment induces the 6-day peak only. Furthermore, phenobarbital induces every mouse with a peak activity at 10 days. It was of interest to determine whether other bilirubin conjugating activities were affected by hydrocarbon treatment which could be crucial to the interpretation of the data which indicated that the hydrocarbon-induced 10-day peak is genetically regulated and associated with the Ah locus. Since bilirubin can be excreted as bilirubin monoglucuronide (BMG) and bilirubin diglucuronide (BDG), it was established by transmethylation of the enzymatic products (using enzymes from 6- and 10-day treated C57BL/6N and DBA/2N mice) and subsequent separation of the bilirubin monomethyl and bilirubin dimethyl derivatives by thin-layer chromatography that the mice synthesize essentially 95% bilirubin monoglucuronide with any enzyme preparation. Furthermore, in collaboration with Dr. I. M. Arias' laboratory, Albert Einstein Medical School, it was shown by high pressure liquid chromatography that the product of a newly described microsomal UDP-glucuronic acid-dependent glucuronoside glucuronosyltransferase is barely detectable using microsomes from any of the aromatic hydrocarbon-treated prototype mice. This enzyme is distinct from UDP glucuronosyltransferase; it utilizes bilirubin monoglucuronide and UDP-glucuronic acid to produce bilirubin diglucuronide.

New purification schemes for separating UDP glucuronosyltransferase(s) utilizing p-nitrophenol, α-naphthol, bilirubin and morphine as substrates are being developed. Because of the high cytochrome P-450 content in microsomal membranes and the apparent low UDP glucuronosyltransferase content in

these same membranes it is very difficult to obtain final preparations of UDP glucuronosyltransferase activity free of cytochrome P-450. By utilize hydrophobic and hydroxyapatite columns which are established purification tools for cytochrome P-450, it appears that UDP glucuronosyltransferases bind and elute from these same columns differently from cytochrome P-450. Furthermore, buffer systems adapted in these purification schemes appear to enhance transferase stability during purification compared to the purification schemes already published for these transferase enzymes. This newly adapted purification scheme appears to generate a transferase preparation which is totally free of cytochrome P-450 which is important to the development of transferase antibodies.

In cell culture the Ah locus apparently regulates the inducibility by aromatic hydrocarbons of a group of genetically-linked structural genes consisting of monooxygenases as well as UDP glucuronosyltransferases. Culture studies with a responsive wild type hepatoma cell line, Hepa-1 C_1C_7, and a nonresponsive mutant cell line derived from Hepa-1 C_1C_7 demonstrate that aryl hydrocarbon hydroxylase (benzo[a]pyrene as substrate) activity and UDP glucuronosyltransferase activity (3-hydroxybenzo[a]pyrene as substrate) are not always induced concomitantly and to the same extent.

Developmental studies in C57BL/6N mice have been undertaken to develop a model for valproic acid hepatotoxicity, as valproic acid has caused significant toxicity and a few deaths in the course of its use as a antiseizure drug. Since valproic acid is uniquely toxic in children, we have attempted to develop a toxicity model in immature animals. It is known that this compound is oxygenated by cytochrome P-450 and glucuronidated by UDP glucuronosyltransferase(s). Our results indicate that valproic acid in 15-day-old C57BL/6N mice has a substantially longer half-life than that in the corresponding mature animal. Furthermore, the 13- to 15-day-old animal after about one week loses all body hair without loss of hair on the head with two daily doses of 125 mg valproic acid/kg body weight which together are equivalent to the daily dose for humans. Older and mature mice do not develop these symptoms.

Significance to Biomedical Research and the Program of the Institute: As discussed previously, drug oxygenations and subsequent glucuronide conjugations are catalyzed by a membrane-bound multistep enzyme complex. These enzymes are important in the process of detoxification and elimination of many normal body constituents and many xenobiotics taken in from our environment in the form of food additives, air pollutants, and medication. There are, as well, diseases of humans ascribed to defects (not well understood) in drug-conjugating enzymes. Examples in pediatrics include neonatal hyperbilirubinemia, Gilbert's syndrome, and the Crigler-Najjar syndrome. Therefore, the development of experimental model systems for understanding better the molecular regulation and neonatal development of UDP glucuronosyltransferase is pertinent to the program of the Institute in the following areas: (1) understanding the mechanisms of "immaturity of drug metabolism" in neonates; (2) providing further experimental models for other human pharmacogenetic defects; (3) elucidating the basic enzymology of glucuronidating drug metabolism in order to gain insight into the development of selective inhibitors and stimulators of these drug conjugating enzymes that are useful for

beneficial regulation of abnormal metabolism peculiar to various human diseases; and (4) understanding metabolism of various drugs in the fetus, premature and the newborn.

Proposed Course of Project: (1) Extend studies on activation of benzo[a]-pyrene phenols to mutagens and reactive metabolites, (particularly the carcinogenic 2-hydroxybenzo[a]pyrene) and the effect substrates for conjugation has on activation of those phenols; (2) further exploration of the genetic difference in response to aromatic hydrocarbon treatment between certain inbred strains of mice to determine substrate preferences for the induced transferase; (3) utilization of different recombinant inbred lines phenotyped for the Ah locus to determine if one genetically regulated UDP glucuronosyltransferase is responsible for the increased conjugating activity for the five or more transferase substrates associated with the Ah locus; (4) examination of urine and bile for glucuronides of labelled exogenous substrates administered to treated responsive and nonresponsive mice; (5) determination of time course for glucuronide and other metabolites of the monooxygenase system, with a comparison of various carcinogenic and noncarcinogenic polycyclic hydrocarbons in the two genetic strains of mice; (6) further characterization of the transferase systems in cell culture with respect to number of different transferases, macromolecular syntheses requirements, and activators and general regulation at the molecular level; (7) determination of bilirubin tolerance in responsive and nonresponsive mouse after hydrocarbon treatment; (8) further examination of the effect of UDP glucuronosyltransferase on bacterial mutagenesis; (9) development of a useful bioassay in cell culture for the quantitation of "breast milk jaundice factor" in human breast milk samples from outpatients and/or inpatients; (10) further purification of UDP glucuronosyltransferase and development of antibodies for enzyme characterization; (11) attempt to develop hybridoma cell lines for monoclonal transferase antibody production; and (12) attempt to develop a hepatotoxicity model for valproic acid in young mice as an aid to understanding valproic acid toxicity in children.

Publications:

1. Malik, N., Koteen, G.M., and Owens, I.S.: Induction of UDP-glucuronosyltransferase activity in the Reuber H-4-II-E hepatoma cell culture. Mol. Pharmacol. 16: 950-960, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00500-02 DP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Hormonal Regulation of Gene Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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John Stevens - Research Institute of the Hospital for Joint Diseases, New York.
Robert Pratt - National Institute of Dental Research

LAB/BRANCH
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12/12	12/12	

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with biochemical and molecular aspects of hormone action during fetal development. Adrenal corticosteroids affect many aspects of fetal development and also have teratogenic effects in genetically-susceptible inbred mouse strains. The glucocorticoid receptor is purified by DNA-affinity chromatography and antibodies to the receptor are prepared for use in determining the chemical structure and function of the receptor.

Project Description:

Objectives: Adrenal corticosteroids regulate many aspects of metabolism and gene expression in the mammalian fetus. The major goal of this project is to understand the mechanism of action of glucocorticoids and to develop a comprehensive understanding of glucocorticoid effects on the developing fetus. The biological effects of glucocorticoids are mediated by a cytosolic receptor protein. This protein has been isolated, and antisera to the receptor have been produced. The antisera provide a unique means for studying glucocorticoid action.

Methods Employed: The cytosolic receptor for glucocorticoids is isolated by DNA-affinity chromatography and polyacrylamide gel electrophoresis. Antisera to the purified receptor are produced in rabbits, and the anti-receptor antibodies are purified. Various immunochemical methods are used to study receptor-antibody reactions including immunoaffinity chromatography and immunofluorescence.

Major Findings: An antiserum to the rat liver glucocorticoid receptor was initially produced in a rabbit in 1979. Only limited amounts of high-titre serum were obtained from this animal, and studies with this material were limited. During the past year, antisera have been produced in three additional rabbits, and relatively large quantities of serum are now available for immunochemical studies. The initial work with the antisera has dealt with basic characterization of the antibodies and determination of their specificity. Although the antisera react best with the rat glucocorticoid receptor, there is substantial cross-reactivity with receptor from other species such as the mouse and human. Studies with mouse and human receptors have been carried out in collaboration with Dr. John Stevens. We have shown that human leukemic cells (circulating and cultured lines) contain glucocorticoid receptors that cross react with the antisera.

The cross reactivity of the antisera with other corticosteroid binding proteins has been studied in collaboration with Dr. Gerald Litwack. We have found that the antisera react with the major form of glucocorticoid receptor (Binder II) but do not react with various proteolytic fragments, or with the major form of renal cortex receptor (corticosteroid Binder IB). In collaboration with Dr. Robert Pratt, we have shown that the antibodies can be used to localize the glucocorticoid receptor by immunofluorescence techniques.

Significance to Biomedical Research and the Program of the Institute: The glucocorticoid receptor is present in extremely low concentrations and has been extremely difficult to purify. The anti-receptor antibody provides a new biochemical tool for isolating the receptor and studying its function in regulating gene expression. Immunochemical methods can be used for developmental studies. Teratogenic effects of glucocorticoids such as the development of cleft palate may involve altered receptor concentrations in genetically susceptible inbred mouse strains. Immunofluorescent localization of the receptor in complex tissues of the fetus should provide important new insights into mechanisms of teratogenesis. Mutations involving the glucocorticoid receptor appear to be responsible for many glucocorticoid resistant leukemias

and lymphomas. The antisera are currently being used to evaluate the receptors in circulating human leukemic cells.

Proposed Course of Project: (1) Molecular biology of the glucocorticoid receptor. Currently used procedures will be modified to permit large-scale purification and characterization of the glucocorticoid receptor. The currently available antibodies will be used in conjunction with other affinity techniques for isolation of biologically "active" receptor (i.e. receptor that retains capacity to bind steroids and chromatin). (2) Production of anti-receptor antibody in cloned "hybridoma" cultures. Mice will be immunized with receptor, and spleen cells from these animals will be fused with myeloma cells in order to derive monoclonal antibodies to the glucocorticoid receptor. (3) Role of glucocorticoids in genetic susceptibility to birth defects (cleft palate). Immunofluorescence studies will be carried out to determine ontogenesis and distribution of the glucocorticoid receptor in the mammalian fetus. Inbred strains of mice differ in susceptibility to glucocorticoid-induced facial defects. These strains will be used to test the hypothesis that differences in receptor concentration affect the response to glucocorticoids. The antisera currently available cross-react with other mammalian species, and it is possible that developmental studies can be carried out in fetal primates. (4) Altered glucocorticoid receptors in murine and human leukemic cells. In collaboration with Dr. John Stevens, we plan to analyze mutant (glucocorticoid resistant) murine and human leukemic and lymphoma cells in order to determine the structure of mutant receptors.

Publications:

1. Hannah, R.M., Simkins, R.A., and Eisen, H.J.: Alpha fetoprotein and albumin synthesis by fetal liver cultured in chemically defined medium. Devel. Biol. 77: 244-252, 1980.

2. Eisen, H.J., Nebert, D.W., and Okey, A.B.: Genetics and the biological response to environmental toxic agents. In: The Chemistry of Environmental Agents as Potential Human Hazards. Washington, D.C., American Chemical Society Press, 1980, in press.

3. Eisen, H.J.: An antiserum to the rat liver glucocorticoid receptor. Proc. Nat. Acad. Sci. U.S.A. 77: 3893-3897, 1980.

4. Litwack, G., Schmidt, T.J., Markovic, R.D., Eisen, H.J., Barnett, C.A., DiSorbo, D.M., and Phelps, D.S.: Activation and DNA binding of the glucocorticoid receptor. In Bresciani, F. (Ed.): Perspectives in Steroid Receptor Research. New York, Raven Press, 1980, in press.

5. Markovic, R.D., Eisen, H.J., Parchman, L.G., and Litwack, G.: Corticosteroid Binder IB is a second glucocorticoid receptor. Biochemistry, in press, 1980.

6. Stevens, J., Eisen, H.J., Stevens, Y.W., Haubenstock, H., Rosenthal, R.L., and Artishevsky, A.: Immunoaffinity chromatography distinguishes between glucocorticoid receptors from corticoid sensitive and resistant mouse lymphoma P1798. Cancer Res., in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00501-03 DP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Non-Invasive Studies of Internal pH and Metabolism of Biological Systems Using Nuclear Magnetic Resonance Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
Developmental Pharmacology Branch

SECTION
Unit on Physical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12/12	12/12	

SUMMARY OF WORK (200 words or less - underline keywords)

The ^{31}P NMR technique enables signals from phosphates in tissues, cells or granules to be monitored directly. The measurement of internal cell pH using ATP_{γ} or P_i is being tested quantitatively and improved probe molecules are being developed to measure ΔpH and trans-membrane potential ($\Delta\psi$) in cell ghosts with intact transport systems. Carbon-13 NMR studies of ^{13}C enriched metabolites are also being pursued.

Project Description:

Objectives: To obtain reliable measurements of internal pH in cells and their components and to understand the processes of membrane transport of ions and protons, and the process of exocytosis. To compare metabolism in normal and diseased cells and to study the processes of phosphorylation or acetylation of important macromolecules in situ.

Methods Employed: High field phosphorus-31 and carbon-13 nuclear magnetic resonance spectroscopy.

Major Findings:

A. The Measurement of Internal pH by ^{31}P NMR: ^{31}P NMR is now widely used as a technique for the non-disturbing measurement of internal cell pH. The value of internal pH has been obtained by reference to a standard calibration curve for P_i or ATP-P_γ using a mixture of ionic components to approximate the cell interior. We have shown that a different calibration curve results for the titration of internal ATP-P_γ in chromaffin granules than is found for the mixture in free solution (Pollard et al., J. Biol. Chem. 254: 1170, 1979). This observation has now been extended to P_i titration curves. Two resolved ^{31}P signals were observed for P_i in the presence of DPL vesicles to which Ca^{2+} was added to the external solution. These signals were attributed to internal and external P_i at the same pH. Both signals titrated with change in external pH, and it was confirmed by the 9-aminoacridine fluorescence method that the vesicles are permeable to protons. Although the differences in chemical shift observed are small they are of the same order of magnitude reported for the resolution of two P_i peaks observed in cells and muscles. The interpretation of such signals, in terms of specific pH compartments, by many studies in the literature may need re-evaluation as a consequence of these results.

Because of these results it was concluded that there is a need for an independent and accurate method to measure internal pH. A new class of pH-probes for use in conjuction with NMR spectroscopy was designed. These are based on monoesters of diphosphonic acids. Since only one phosphonic acid residue titrates at physiological pH, the difference in chemical shift values between the two phosphorus resonances originating from the same molecule will be an accurate monitor of pH. By inbedding these lipophobic molecules into synthetic liposomes of reconstituted membrane residues one can measure the internal pH nondestructively and in a time-resolved manner.

It should also be possible to measure membrane potentials simultaneously with internal pH or trans-membrane pH gradient by the ^{31}P NMR method. Bis-biphenylene phosphoium tris-biphenylene phosphate salt, which contains both positively and negatively charged phosphorus atoms, should be applicable for direct measurements of potential difference across the membrane of closed vesicles and liposomes. It is expected that these highly lipophobic ions will equilibrate on either side of the membrane according to its potential difference.

Synthesis of both ΔpH and $\Delta\psi$ probes are currently in progress in this laboratory.

B. The work on chromaffin granules, in collaboration with Harvey Polland's laboratory, is being continued. Our tentative results indicate the intimate interrelationship of the anion-transport site and proton translocating activity.

Work was initiated on the reconstitution of vesicles ("ghosts") of chromaffin granules. Our preliminary results indicate that these vesicles contain active proton-translocating ATPase which we intend to study with a variety of methods, including NMR.

C. Metabolism of protozoa Euglena gracilis grown under photosynthesizeing conditions is being investigated in collaboration with Ellis Kempner's laboratory. Unusually high quantities of inorganic polyphosphates in this organism suggest some major diviations from the usual metabolic pathways. We are planning to provide more detailed answers to this question by following the metabolism with ^{13}C enriched metabolites. In order to carry out such NMR studies on micro-organisms some time has been expended to design an appropriate NMR cell, so far with limited success.

D. The work on mouse Le cells treated with interferon has been discontinued because it proved impossible to obtain reproducible results with different cell preparations.

Significance to Biomedical Research and the Program of the Institute: (1) Prior attempts to measure internal cell pH have been difficult and intrusive. ^{31}P NMR has provided a nonintrusive method, but quantitation of the method is difficult. With the methods under development it should be possible not only to obtain accurate values of internal pH, but a greater understanding of ion transport through membranes and of the mechanism of exocytosis. (2) Studies of cell metabolism using NMR methods should provide insight into subtle aspects of metabolic pathways which are of general significance.

Proposed Course: (1) Studies on chromaffin granules will be continued utilizing the information we now have to study factors affecting changes in ΔpH and the process of exocytosis. (2) Studies on reconstituted membrane ghosts, containing active proton transport systems, will be made with the specially designed ΔpH and $\Delta\psi$ probes. (3) Metabolic studies on selected micro-organisms will be undertaken when the system may provide new information.

NPMB, NICHD Annual Report--October 1, 1979 through September 30, 1980

The Neonatal and Pediatric Medicine Branch has continued its broad research program in both clinical and basic approaches to the diagnosis, treatment and fundamental mechanisms of diseases of infants and children. Included in the program are genetic diseases, inborn errors of metabolism, disordered nutritional states, congenital malformations, endocrine and metabolic disturbances, as well as the effects of disorders of maternal origin on the neonate. This group also studies the normal biological mechanisms of infant and child development.

Currently, there are three Sections and 2 units actively carrying on these research projects. These include: 1) the Section on Developmental Biology and Clinical Nutrition with its units of Endocrinology and Neonatal and Pediatric Mineral Metabolism; 2) the Section on Human Biochemical and Developmental Genetics; and 3) the Section on Gastroenterology and Nutrition. An overview of each will be presented in brief.

I. The Section on Developmental Biology and Clinical Nutrition has ongoing investigations in three distinct areas: a) the developmental and hormonal mechanisms controlling phospholipid biosynthesis in various fetal and adult organs; b) determining the role of the central nervous system in the pathogenesis of massive obesity with or without the Prader-Willi syndrome, and c) the basic mechanisms involved in the diagnosis, treatment and pathogenesis of a number of metabolic disturbances including glycogen storage disease and hypoglycemia with or without hyperinsulinemia. The Endocrinology Unit, (A) is studying the mechanisms of early sexual development with a primary emphasis on improving methodology to measure Mullerian Inhibiting Substance (MIS). Other areas of study include the role of LH-RH in the development of the hypothalamic-pituitary-gonad axis. Collaborative studies have also been undertaken to explore the possibility of unusual thyroid hormone dysfunction in patients with cystinosis. The Neonatal and Pediatric Mineral Metabolism Unit (B) continues their research effort on metabolic and nutritional aspects of growth and development, mineral metabolism in infancy and childhood both in health and disease and endocrine functions occurring between conception and the attainment of adulthood.

a) The developmental studies of phospholipid biosynthesis in fetal lung have concentrated this year in three areas. 1) Insulin stimulates choline kinase activity in fetal lung explants. This effect appears to be mediated by specific insulin receptors, as the amounts of enzyme activation is proportional to the affinities of various insulins to the insulin receptor. Insulin receptors have been detected in lungs of fetal rats as early as 15 days gestational age. When the insulin binding data was subjected to Scatchard analysis, classical curvilinear plots, typical of insulin receptors, were obtained. 2) Morphine sulfate was found to inhibit phosphatidylcholine (PC) biosynthesis in fetal rat lung explants in a dose dependent manner. Naloxone did not reverse this effect. 3) A mixed fetal rat lung cell culture has been established to study biosynthesis and secretion of PC.

b) The studies in childhood obesity have continued. The basic approach has been to ascertain the circadian pattern of the obese child and to repeat these studies when normal weight is attained. In the past year the melatonin

rhythm has been added. Attempts to evaluate the level and potential role of β endorphins and bombesin have been unsuccessful for technical reasons. Only preliminary data at this point are available for a limited number of children who have been tested after regaining normal weight. Studies are projected for the determination of responsivity to melatonin for various light intensities in obese vs normal weight. Studies to discern the reason for the differential in the rate of weight loss in males and females will be undertaken. Evaluation of brown fat activities in family members will be pursued when obese and normal weight members exist. Studies of the difference in handling glucose, fatty acids and/or leucine labeled with stable isotopes will be undertaken.

The ongoing investigation of glycogen storage disease, Type II, has progressed from the purification of human placental lysosomal (1-4) alpha-glucosidase to the production of antibodies to the renal and placental enzyme for urinary assays applicable to family studies. The purified enzyme will also be used to study the role of alpha-glucosidase in normal glycogen metabolism.

In addition, preliminary work has begun to develop techniques to study in vivo glycogen metabolism in obese and glycogen storage disease patients utilizing stable isotopes.

c) Methods to diagnose specific metabolic disorders such as type II glycogen storage disease, receptor defects in hypoglycemia and hyperglycemia and substrate such as ketone bodies, lactate, pyruvate and amino acids have been set up in order to better understand the pathophysiology of these disorders. Insulin receptor sites on red cells in a variety of disorders of carbohydrate metabolism have been measured throughout development and in patients with hypoglycemia both on and off diazoxide treatment.

All these studies are designed to determine and understand the basic mechanisms underlying normal growth and development. Animal models are being established to further investigate the mechanisms of the clinical observations.

(A) The unit on Pediatric Endocrinology continues its studies in the following areas: 1) The development of the hypothalamic-pituitary-testicular axis; 2) neuroendocrine secretion in primates; 3) abnormalities in endocrine function in cystinosis.

1) Both bovine and rat Sertoli cell culture have been studied. We have demonstrated autoregulation of FSH response in rat Sertoli cells including analysis of FSH receptors, FSH stimulation of cyclic AMP and aromatization of testosterone to estradiol. In the bovine Sertoli cells, unlike the rat cells, we have shown FSH stimulates an increase in the enzyme, ornithine decarboxylase. Indirect evidence suggest the Sertoli cell is the source of Mullerian inhibiting substance. Using a high titer antiserum to luteinizing hormone-releasing hormone (LHRH), we have studied effects on androgen binding protein in the developing rat. In addition we have done further studies on the mechanism of the previously shown permanent alterations in the hypothalamic-pituitary-testicular axis. Our laboratory continues its efforts to relate these problems to intersex abnormalities and their relationship to Mullerian inhibiting substance secretion in clinical medicine.

2) A primate model for neuroendocrine evaluation over a short period has been established. We have demonstrated transient alterations in primates treated with different doses of cranial irradiation. Other studies are in progress examining the effect of neuroplasmacologic agents on primate neuroendocrine secretion.

3) Studies in cystinotic have demonstrated pituitary resistance to thyroid hormones. Further in vivo and in vitro studies are in progress in order to better understand the mechanisms involved.

All these studies are designed to better understand developmental endocrine problems in normal and abnormal sexual development.

(B) In the Unit on Neonatal and Pediatric Mineral Metabolism, interests center on metabolic and nutritional aspects of growth and development, mineral metabolism in infancy and childhood (both in healthy children and during various disease states), and endocrine functions occurring between conception and the attainment of adulthood. Having shown the utility of stable isotopic tracer techniques to study calcium dynamics in infancy using a highly sophisticated mass spectrometry, we have developed a practical analytical method using a widely available quadrupole mass spectrometer making these techniques more generally accessible. Attempts to measure levels of iron, zinc, and copper in extremely small samples revealed striking matrix interference effects which can now be accounted for; accordingly, new tests for these essential trace elements in infancy were developed. Time series analysis of daily urinary gonadotropin excretion rates in a girl during the transition into the earliest stage of puberty disclosed a monthly cyclic pattern which changed in shape as pubescence began and a stable 13 day cyclic pattern not recognized previously in hormones of reproduction. Work will continue on characterizing the nutritional status of children with disorders effecting the assimilaton and utilization of key dietary minerals (calcium, iron, zinc, copper, etc.), and urinary gonadotropin data from other girls and from boys will be analyzed for changes in cyclic patterns occurring during puberty.

II. The Section on Human Biochemical and Developmental Genetics continues both its basic science and clinical investigation of cystinosis and other defects in sulphur amino acid metabolism. Other current areas of research include amino acid transport, lysosomal structure and function, role of amino acids and fetal and neonatal nutrition and the biochemistry of early embryonic development. Continuing investigations of glutathione metabolism both in animals and in man have generated new approaches to the therapy of chronic hemolytic anemia in red cell enzyme deficiencies. Ongoing clinical investigations include the evaluation of cysteamine in reducing the storage of cystine in patients with cystinosis, and a collaborative world wide project to study the effectiveness of high doses of vitamin E (alpha tocopherol) in preventing the chronic hemolysis in glucose-6-phosphate-dehydrogenase deficient patients. both of these investigations are ongoing and definitive results will be forthcoming within the near future.

Major efforts continue on the incorporation of human genes into mammalian cells with the use of liposomes (lipochromosomes). Feasibility of producing lipochromosomes and using them to transfer X-linked genes has been previously reported. Work also continues on the development of a selective system for cystinotic cells in culture and demonstrating differences that exist between normal and cystinotic cells. The section actively pursues study of the effects of various drugs and biochemical pertubations in reducing the cystine content of cystinotic cells as well as studying detailed metabolic pathways and mechanisms in its pathophysiology.

A new contribution has been the demonstration that specific HLA types occur more frequently in patients with the recessive genetic disorder cystinosis than in a comparable normal population. These data represent the first demonstration of this relationship and will be extended to other genetic diseases as well.

In summary, studies will continue to focus in two areas. First the metabolism of amino acids and especially sulphur containing amino acids and peptides and inborn errors of metabolism involving these compounds. The second area will be on amino acids as they relate to nutritional problems and intrauterine and post natal growth.

III. The Section on Gastroenterology and Nutrition has been studying intestinal epithelial cell migration and differentiation and the nutritional modifications of gene activity and expression in developing and adult mammals. With respect to epithelial cell migration, our efforts have been focused on the establishment of a primary cell culture system which will allow us to define those cell surface components which are responsible for cell attachment to the basement membrane. In addition, an intestinal organ culture system is being developed as an *in vitro* model of cellular locomotion along the crypt-villus axis, so that the roles of protein synthesis and hormonal influences on intestinal cell migration can be investigated. We are also continuing our ongoing research into the control of sucrase/isomaltase synthesis and the mode of incorporation of the complex into the intestinal brush-border membrane. Primary cultures of rat pancreatic exocrine cells are being established to elucidate the role of nutrition in the modulation of gene activity and expression. Initially, studies will focus on the mechanism of adaptation of rat amylase in isolated cells and intact pancreas to carbohydrate in the media or the diet.

In conclusion, the clinical activities of the Neonatal and Pediatric Medicine Branch have expanded significantly over this current fiscal year. The establishment of a pediatric component to the weekly Inter-institute Endocrine Clinic has attracted patients from around the world. Research protocols have been generated based on these new opportunities. In addition, an Inter-institute Genetics Clinic spearheaded by this Branch has been initiated and is also beginning to generate patient-referrals and protocols.

The short tenure due to fire of the expanded in-patient service of 23 beds was characterized by almost full occupancy at all times. Patients from NICHD accounted for 45 to 50 percent of the in-patient population. Although our clinical facilities are strictly limited at this time, the Clinical Program continues to thrive.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00126-06 NPMB

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Developmental Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

R.E. Ulane, Ph.D.	Chemist, DBCN Section	NPMB, NICHD

Other Investigators:

M. Cornblath, M.D.	Acting Chief, NPMB	NPMB, NICHD
J. Graeber, M.D.	Clinical Associate, DBCN	NPMB, NICHD

COOPERATING UNITS (if any)

J. Roth, M.D.	Chief, DB	NIAMDD

LAB/BRANCH

Neonatal and Pediatric Medicine Branch

SECTION

Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The control of fetal lung surfactant production is being studied by assessment of its principal phospholipid component, phosphatidylcholine. Choline Kinase has been established as a rate controlling step in PC synthesis. Insulin was demonstrated to activate this enzyme in a specific and dose - dependent manner in fetal lung explants. Multiplication stimulating activity also activated the enzyme. Specific insulin receptors were demonstated in fetal lung tissue as early as 15 days gestational age and exhibited classical curvilinear plots when binding data was subjected to Scatchard analysis. A mixed fetal lung cell culture has been established which provides a system for studying the control of the biosynthesis and secretion of disaturated phosphatidylcholine.

Project Description:

Objectives: Studies on organ development have focused on fetal and neonatal lung as related to the respiratory distress syndrome (RDS) of human neonates. This disorder occurs in low birth-weight infants, is characterized by progressive pulmonary atelectasis, and accounts for greater mortality and morbidity than any other neonatal disorder or pediatric pulmonary disease. A decrease in the concentration of surfactant, composed primarily of disaturated phosphatidylcholine (PC) and a smaller but important amount of phosphatidylglycerol, (PG) are closely associated with the development of RDS. Thus, phosphatidylcholine and phosphatidylglycerol biosynthesis and secretion are essential to the development of a mature and functional pulmonary system. We have addressed ourselves, therefore to the following objectives: 1) To investigate the control of phosphatidylcholine and phosphatidylglycerol biosynthesis and secretion during fetal lung development. 2) To investigate the effects of diabetes, nutrition and drug addiction during pregnancy on phospholipid synthesis in fetal lung. 3) To establish a mixed fetal lung cell culture capable of demonstrating the biosynthesis and secretion of disaturated phosphatidylcholine.

Methods Employed: PC biosynthesis is measured using isotopic substrates, extraction of labelled phospholipids, and separation into individual species utilizing thin layer and paper chromatography techniques. Fetal rat lung explants are utilized to study PC synthesis in intact cells. Mixed fetal rat lung cell cultures were obtained by trypsinization of fetal lung explants and subsequent plating in cell culture dishes. Type II pneumocyte populations in the cell cultures are assessed by fluorescent staining of lammeller bodies with phosphene dye. Viability of cells is assessed by ^{14}C-Arachidonic acid incorporation into cellular phospholipids. Fetal rat lung homogenates are employed to examine individual PC biosynthetic enzymes.

Major Findings: Through the use of a specific inhibitor, hemicholinium-3, it was found that choline kinase was a rate controlling step in PC synthesis. Incubation of fetal lung explants with insulin was found to increase choline kinase specific activity in a dose-dependent manner. Multiplication stimulating activity also increased choline kinase activity. The maximal stimulation of choline kinase occured in fetal explants from rats at 19 days gestational age. The increase in choline kinase activity could be blocked by the addition of either cycloheximide or actinomycin D. Glucagon, cortisol and prolactin in combination with insulin or alone had no effect on choline kinase activity. Specific insulin receptors have been detected in fetal rat lungs as early as 15 days gestational age. The insulin binding data from this fetal tissue displays a classical biphasic curve due to negative cooperativity when subjected to Scatchard analysis.

The incorporation of ^{14}C-choline into phosphatidylcholine was inhibited in fetal lung explants incubated in the presence of morphine sulfate for 24 hours. This inhibition was dose dependent, with maximal inhibition occurring at 10^{-5} M morphine sulfate. Levorphanol had no effect on PC synthesis. Naloxone did not reverse the effects of morphine on inhibition of PC synthesis but actually contributed to inhibition of its synthesis. Naloxone alone had

no effect on PC synthesis between 10^{-4} and 10^{-9} M. The presence of opiate receptors in fetal lung tissue could not be detected, although their presence has not been ruled out. In contrast to the effects on organ explants, daily morphine sulfate injections of pregnant rats starting at 9 days after mating stimulated PC synthesis in lung slices from Caesarian delivered rat fetuses at 16 and 17 days gestational age when compared to controls from mothers injected with saline.

A mixed fetal rat lung cell culture has been established for the purpose of studying disaturated phosphatidylcholine biosynthesis and secretion. The population of type II pneumocytes is about 20% of total cells present. This cell culture has a high capacity to incorporate ^{14}C-arachidonic acid into phosphatidylcholine and to a lesser degree into phosphatidylethanolomine, phosphatidylserine and phosphatidylinositol. Methods have been established to study the hydrolysis of arachidonic acid from phosphatidylcholine. Calcium ionophor 23187 has been found to stimulate this hydrolysis.

Significance to Biomedical Research and the Program of the Institute: In order to study the metabolic disturbances leading to RDS, basic features of phospholipid metabolism in lung must be identified, particularly those factors which play a role in PC biosynthesis. We have taken the approach of looking into several disorders during pregnancy, including diabetes and drug addictio , that many alter pulmonary maturation in the fetus.

The observation that maternal diabetes is associated with an increased incidence of RDS has led us to look into the relationship between insulin levels and the control of phospholipid synthesis. The observation that choline kinase is a rate controlling step in PC synthesis is important in view of its demonstrated sensitivity to insulin levels. The observation that insulin receptors are present in fetal lung also makes these findings significant in studying the specific effects of insulin on PC synthesis.

The direct effects of morphine on fetal lung tissue PC biosynthesis appear to be non-specific and not mediated by opiate receptors. In view of this observation the stimulation of fetal pulmonary PC biosynthesis when pregnant rats are given daily doses of morphine appears to be a secondary effect of the drug.

Most studies involving the control of disaturated PC levels in fetal lung have been concerned with mechanisms involved with the biosynthesis of PC and not its subsequent secretion from Type II pneumocytes. The mixed fetal lung cell culture that has been established will allow for a detailed study of what stimulates disaturated PC secretion from these cells.

Proposed Course of Research: Insulin receptor levels will be measured as a function of gestational age. The receptors will also be characterized to determine if their binding constants change as a function of gestational age.

The effects of morphine sulfate on phosphatidylcholine synthesis in fetal lungs will be examined for a change in lamellar body content when treated with morphine sulfate. The lack of effect of naloxone and levorphanol,

while morphine sulfate demonstrates a dose dependent inhibition of PC synthesis will be examined.

The effects of various activators of phospholipase A_2 and arachidonic acid hydrolysis will be investigated. This enzyme may be responsible for the "remodeling" of unsaturated PC to yield disaturated PC, a primary component of sufactant. With the mixed fetal lung cell cultures, the secretion of disaturated PC will be examined. The role of arachidonic acid and its metabolites in the synthesis and secretion of disaturated PC will also be studied.

Publications:

1. Ulane, R.E., Ulane, M.M.: The effects of insulin on choline kinase activity in cultured rat liver cells. Life Sciences, 26: 2143-2149, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00127-06 NPMB

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Developmental Endocrinology and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

J.W. Hansen	Sr. Investigator	NPMB, NICHD

Other Investigators:

B. Corden	Clinical Associate	NPMB, NICHD
Nancy Vieira	Biologist	NPMB, NICHD

COOPERATING UNITS (if any)

H.J. Hoffman, Biometry Branch, NICHD
J.D. Schulman, NICHD

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Section on Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

XX

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying metabolic changes which occur as an individual develops from a fetus to a mature adult. We are currently focusing on two areas: 1) monthly gonadotropin cycles in premenarchial girls and pubescent boys utilizing radioimmunoassay methods for measuring urinary luteinizing hormone and urinary follicle stimulating hormone and analyzing the data with autoregressive computer techniques, and 2) mineral metabolism in the rat, monkey fetus, and human newborn. Calcium kinetics are studied in the patients using stable calcium isotopes analyzed in the laboratory by mass spectrometry. Physiological effects of calcium are studied by measurements of ionized calcium in the blood. Trace element (zinc, copper and iron) analyses are performed by atomic absorption spectrophotometry as part of our studies of nutritional aspects of mineral metabolism in infancy and childhood.

PHS-6040

Project Description:

Objectives: We are studying kinetics of calcium and trace element metabolism in the period around birth to understand better and to aid the newborn infant in its adjustment to extrauterine life. A special care nursery has been established to enable these and other clinical projects.

Methods Employed: Computer analysis of the data using autoregressive filtering techniques; urinary hydroxyproline and serum alkaline phosphatase, calcium, phosphorus, magnesium, zinc, copper, iron, total protein, albumin, pH, and ionized calcium measurements; calcium kinetic studies using stable isotopes and noninvasive urinary collection techniques for sample acquisition in newborn infants; mass spectroscopy of calcium isotopes; and radioisotope kinetic studies of calcium and trace element metabolism in laboratory animals.

Major Findings: Having shown that newborn calcium metabolism differs markedly from adult calcium metabolism, we used radiocalcium tracers to study possible influences of various hormones and nutrients on endogenous fecal excretion in the rat. This was possible only because endogenous fecal calcium excretion bears the same relationship to urinary excretion in the rat as it does in the newborn human. Vitamin D and calcitonin were found to improve calcium retention by increasing absorption, decreasing excretion and decreasing bone calcium resorption. Paradoxically, calcitonin increases endogenous fecal calcium loss. Medium chain triglycerides were also shown to increase fecal calcium loss and to decrease calcium retention when compared to a fat free diet, though this effect is probably less than a normal fat intake would produce.

Micromethods have been developed using graphite furnace atomic absorption to determine iron, zinc and copper. These techniques require less than 10 microliters of sample and thus make possible studies of trace elements in small newborns. Using these methods, normal adult zinc levels are 0.87 ± 26 ug/ml and copper are 0.99 ± 0.45 ug/ml. The iron technique has been used to begin work on developing a laboratory animal model for hemosiderosis and to find a good way to give iron by injection.

Using the statistical techniques of autoregressive filtering and frequency analysis on data collected for a period of one year from a prepubertal girl, we have confirmed the presence of monthly gonadotropin cycles and disclosed, for the first time, the presence of 13 day cycles.

Significance to Biomedical Research and the Program of the Institute: During the third trimester of intrauterine development, calcium and trace elements are accumulated in the rapidly growing fetus. With delivery, the newborn is removed from a rich supply of these nutrients via the placenta and is susceptible to deficiencies, hypocalcemia, and seizures. This is particularly true of the premature infant. The results of our studies should provide a rational basis for diagnosis and treatment of these infants with nutritional and metabolic problems relating to calcium and trace elements. Studies on developmental endocrinology and metabolism in the period around birth have a direct

bearing on the mission of the Neonatal and Pediatric Medicine Branch and of the NICHD and seek a better understanding of the process of normal growth and development and of the derangements accompanying various disorders.

Proposed Course of Project: Our research is progressively placing more emphasis on endocrinology and metabolism around the time of birth with initial studies being directed toward calcium control and metabolism in the fetus and newborn. We are also investigating trace element metabolism in the newborn as another aspect of developmental metabolism which requires elucidation to improve the nutrition of premature infants. The unique techniques we are using are also being extended to other children with disorders of mineral metabolism. This will give us a better understanding of disease processes and how we can more effectively treat them. We will also complete our analysis of samples previously collected to investigate gonadotropin excretion patterns during pubescence which is disclosing new information on the process of sexual maturation.

Publications:

1. Davis, S.E., Hansen, J.W., Bhatnagar, A.S. and Loriaux, D.L.: Timed 12 hour specimens in outpatient estriol monitoring. J. Ob. Gyn. 55: 105-108, 1980.

2. Yergey, A.L., Vieira, N.E., and Hansen, J.W.: Isotope ratio measurements of urinary calcium with a thermal ionization probe in a quadrupole mass spectrometer. Anal. Chem. In press.

3. Corden, B.J., and Hansen, J.W.: Ferric acetohydroxamate, $Fe(AHA)_3$, a potential parenteral iron supplement. Ped. Res. 14: 465, 1980.

4. Corden, B.J., Schulman, J., and Hansen, J.W.: Hemosiderosis in rats using ferric acetohydroxamate, $Fe(AHA)_3$. A new model for rapid loading, chelating and toxicity studies. Ped. Res. 14: 465, 1980.

5. Yergey, A.L., Vieira, N.E., and Hansen, J.W.: Calcium isotope ratios by a thermal ionization probe in a quadrupole mass spectrometer. Ped. Res. 14: 584, 1980.

6. Lim, M.O., Mukherjee, A., and Hansen, J.W.: Dysplastic cutaneous osteomatosis: a unique case of true osteoma. Arch. Derm. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00131-06 NPMB

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Human Biochemical Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

J.D. Schulman	Head, Section HBDG	NPMB, NICHD

Other Investigators:

E.J. Butler		
D. Towne		
W. Nelson		
R. Steinherz		
V. Soutter		
J. Chemke	Visiting Scientist	NPMB, NICHD
Y.T. Chen		
W. Rizzo		

COOPERATING UNITS (if any)

SEE ATTACHED

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Human Biochemical and Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
11	10	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS X ☐ (b) HUMAN TISSUES X ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our studies have concerned the metabolic errors of glutathione synthesis and degradation (glutathionuria, glutathione synthase deficiency and gamma glutamyl-cysteine synthase deficiency), glucose-6-phosphate dehydrogenase deficiency in which the capacity for glutathione reduction is decreased, cystinosis, homocystinuria, phenylketonuria, non-ketotic hyperglycinemia, galactosemia and adrenal leukodystrophy. We have been interested in the development of newer forms of treatment and diagnosis for a number of these metabolic disorders and in understanding the mechanisms of disease production and related normal processes. A number of specific treatments are under investigation. Investigations have also been undertaken to explore the possibility of transfering human genetic material into mouse cells with the use of lipochromosomes. Some studies of the growth and metabolism of cultured amniotic fluid cells have also been undertaken in attempts to improve techniques for prenatal diagnosis. Animal models of human genetic diseases are also investigated.

COOPERATING UNITS:

L. Boxer, University of Indiana

J. Oliver, University of Connecticut

R. Berlin, University of Connecticut

P. Donahoe, Harvard

S.H. Mudd, NIMH

F. Tietze, NIAMD

A.D. Patrick, London (Inst. C.H.)

S. Kaufman, NIMH

S. Milstein, NIMH

C. Bartsocas, University of Athens, Greece

S.P. Spielberg, Johns Hopkins University

L. Corash, CC, NIH

F. Oski, Syracuse University

A. Eidelman, Jerusalem, Israel

J. Robbins, NIAMDD

J. Barranger, NINDS

J. Avigan, NHLBI

H. Moser, Johns Hopkins

D. Mattison, NICHD

K. Mittal, NBS

M. Sheetz, University of Connecticut

S. Moses, Beersheva, Israel

N. Bashan, Beersheva, Israel

B. White, NIAMDD

S. Wachtel, Sloan Kettering

J.L. Simpson, Northwestern

A. Mukherjee, NICHD

S.I. Goodman, University of Colorado

J. Schlesselman, NICHD

J.A. Schneider, UCSD

J. Thoene, University of Michigan

Project Description:

Objectives: The goals of the project are as follows: (1) to elucidate the basic biochemical defects responsible for selected inborn errors of metabolism in man; (2) to explore the secondary biochemical and physiological derangements caused by the primary metabolic defect; (3) to develop new or improved methods of treatment; (4) to develop new or improved methods of diagnosis, both antenatal and postnatal; (5) to use genetically abnormal cells and patients to expand understanding of normal human biochemistry, physiology, and development; (6) to see if human genes can be introduced into genetically deficient cells using lipid enclosed chromosomes and to address questions of gene (especially X-chromosome) function in this system. We have chosen to focus primarily on derangements of sulphur amino acid metabolism and secondarily on other disorders of amino acid, sugar, and lipid metabolism and on selected derangements of lysosomal function.

Methods Employed: The principal methods employed are: (1) cell culture techniques, (2) spectrophotometric, radiochemical, and fluorometric enzyme assays, (3) differential ultracentrifugation (4) quantitative ion exchange chromatography of amino acids in physiological fluids, including cellular extracts, (5) radioisotopic measurements, (6) light, phase contrast, and electron microscopy, (7) column and thin-layer chromatography, (8) starch and polyacrylamide gel electrophoresis, (9) osmometry, (10) histochemistry, (11) determinations of red cell and platelet survival after tagging with radioactive chromate (12) liposome technology and (13) dietetic treatment of animals.

Major Findings: Continuing investigations of glutathione metabolism in cultured human cells and in animals have permitted certain observations regarding the role of these enzymes and glutathione itself in biological processes, in addition to the large number of conclusions summarized in earlier project descriptions.

Study of cells from patients deficient in gamma-glutamyl transpeptidase activity, as well as *in vivo* studies of one such patient, constitute the strongest evidence so far available that this enzyme is not, as previously believed, a significant mediator of amino acid uptake in a variety of human cell types. We are now examining whether such patients have defective capacity to excrete cystine in response to lysine loads, which would be predicted if urinary cystine is derived from renal glutathione catabolism.

Glutathione synthase deficient patients have been shown, in contrast to earlier reports, to have normal intracellular amino acid levels in erythrocytes and leukocytes, and in serum.

Successful correction of reduced intracellular glutathione content and of excessive the cells of patients with glutathione synthase deficiency has been oxoproline production in achieved *in vitro* using a mixture of serine and borate, which inhibits gamma-glutamyl transpeptidase activity.

We have demonstrated that the cells from patients with reduced glutathione synthase activity are abnormally susceptible to oxidative damage as demonstrated by a shortening of red cell survival and a susceptibility to damage and microtubule dissociation in leukocytes during phagocytosis. Both the red cell and leukocyte abnormalities are partially and in some cases wholly correctable after the patients have received large doses of the antioxidant vitamin E for several months.

Related studies on the phenomenon of capping in leukocytes from patients with abnormalities of glutathione synthesis have helped to define the role of oxidized glutathione in microtubule assembly and disassembly.

Since glutathione may be involved in the deiodination of the thyroid hormones we are testing whether liver cells with reduced glutathione content have abnormal metabolism of such compounds. It appears that they do only if the oxidation state, not concentration, of glutathione is reduced. In these investigations glutathione depletion and oxidation using cystine starvation, diamide, and other techniques are being utilized.

Since patients with glucose-6-phosphate dehydrogenase deficiency have a reduced capacity to regenerate reduced glutathione after oxidative stress, we have explored the possibility that the hemolytic anemia associated with this disorder might be improved by treatment with high doses of vitamin E. The rate of hemolysis in patients with chronic hemolyzing glucose-6-phosphate dehydrogenase deficiency is not usually reduced with vitamin E treatment. The hemolytic anemia of glutathione synthase deficiency is improved consistently.

We have also demonstrated that the mild chronic hemolysis in Mediterranean type glucose-6-phosphate dehydrogenase deficiency is significantly reduced by high dose vitamin E treatment in a population of Greek subjects. Significant increases in hematocrit and hemoglobin levels, reductions in reticulocyte count and prolongations of red cell survival were documented. It was observed that G6PD deficient individuals have significantly lower vitamin E levels than suitable controls. Further investigations in Israel have confirmed that G6PD deficiency is associated with mild hemolysis and that reduced vitamin E levels are characteristic of such subjects.

In investigations currently underway, we are examining if vitamin E treatment will reduce neonatal hyperbilirubinemia secondary to glucose-6-phosphate dehydrogenase deficiency or whether vitamin E administration will reduce the frequency and severity of the acute hemolytic crises responsible for the major morbidity and mortality in this widely prevalent genetic disease.

The effects of β-carotene, another anti-oxidant, in chronic severe G6PD deficiency are being examined in other clinical trials.

Studies are underway exploring the susceptibility of glucose-6-phosphate-dehydrogenase deficient red cells to oxidative stresses <u>in vitro</u> (such as

retinol-peroxide or aspirin-peroxide). The phospholipid membrane composition of G6PD and normal cells is also being compared under baseline conditions and during conditions of oxidative stress. The mechanisms of destruction of red cells in G6PD deficiency have also been examined with the use of lectins and an increased agglutinability by Concanavalin A as well as increased binding of the C_3 component of complement has been observed in G6PD deficient red cells.

Alterations in the activity of the enzymes regulating the synthesis and degradation and glutathione have been demonstrated at different time points during fibroblast growth in vitro. These enzyme changes predictably alter intracellular glutathione content during cell growth.

Investigations of cystinosis continue to be a primary interest in our laboratory. A number of significant observations on this disease have been made.

A selective system has been developed for cystinotic cells in vitro based on the remarkable resistance of these cells to high concentrations of extracellular cysteine.

Current clinical trials with the use of cysteamine and phosphocysteamine have demonstrated an in vivo reduction in leukocyte cystine content to near the normal range. Initial clinical observations on renal function are promising but not yet conclusive.

In additional studies we have observed that patients with cystinosis may suffer from frank or compensated hyhpothyroidism, and also that a regulatory defect involving the pituitary-thyroid axis exists in this disorder. Increased pituitary resistance to thyroid hormone is suggested by the available data. We are presently examining in more detail the response of the pituitary gland to graded doses of exogenous thyroid hormone in cystinosis.

Studies on the enzyme pantetheinase, a cysteamine generating enzyme, demonstrated its presence in human tissues for the first time and documented that pantetheinase deficiency is not responsible for cystinosis; in addition it has been demonstrated that a deficiency in endogenous cysteamine production cannot account for the increased intracellular storage of cystine in this disease. In additional studies on cystinotic and normal leukocytes, we have demonstrated that the glutathione metabolizing enzyme, gamma glutamyl transpeptidase is a natural component of lysosomes as well as the plasma membrane and the characteristics of this enzyme in cystinotic lysosomes are normal.

We are presently examining the permeability of isolated cystinotic and normal lysosomes to a variety of amino acids including cystine in efforts to establish whether the lysosomal storage of cystine is due to altered permeability characteristics of the lysosomal membrane. These studies are being done with labelled amino acid methylester substrates as described for rat liver lysosomes by Reed.

We have uncovered some new aspects of the population genetics of cystinosis. The large excess of males over females observed in this disorder is not predicted by classical autosomal recessive inheritance. A striking linkage disequilibrium between cystinosis and the HLA antigen system has been demonstrated: HLA A3 and B7 are associated with cystinosis substantially more frequently than would occur by chance. Despite these associations of certain HLA types with cystinosis family studies have demonstrated conclusively that the cystinosis gene is not closely linked to the HLA locus on chromosome 6.

Our interest in genetic diseases related to cystinosis has led us to the description of a new genetic entity which resembles cystinosis clinically, is more severe, but is not characterized by increased intracellular cystine storage; there is a unique combination of renal pathology and clinical symptomatology in this entity which has been observed in siblings.

Work is also continuing on non-sulphur amino acid inborn errors of metabolism. A new form of phenylketonuria due to decreased intracellular biopterin has been identified. A new in vivo assay for phenylalanine hydroxylase activity has been developed and tested. The assay involves the administration of minute amounts of tritiated phenylalanine to patients under an approved protocol. This approach may lead to elimination of the need for liver biopsy in certain types of hyperphenylalaninemic patients and may improve heterozygote identification.

In laboratory animals, studies related to the disease non-ketotic hyperglycemia have been pursued. We have completed a series of investigations on glycine entry into neonatal and adult rat neural tissue and have examined the effect of benzoate on enzymes which conjugate it with glycine, and on the clearance of glycine from various tissues including brain, in the hyperglycinemic rat. These studies enhance our understanding of possible disease mechanisms and approaches to treatment in this very serious disorder, and have confirmed an exceptional sensitivity of the neonatal brain to glycine toxicity.

A major new area of interest has involved the X-linked disorder, adrenal leukodystrophy. We have demonstrated that there is a significant elevation of serum C26 fatty acids in these subjects and have developed an approach to diet therapy based on a restriction of hexacosanoic acid in the food intake of affected patients. Studies are being pursued on determining the contribution of endogenous versus exogenous C26 to the long chain fatty acids stored in patients and in animal models for adrenal leukodystrophy. Fibroblasts from patients, heterozygotes and normal subjects are being investigated using tritium release assays and carbon dioxide evolution from labeled precursors to determine whether there is a defect in long chain fatty acid catabolism in this lethal genetic disease.

We have also undertaken investigations of the effects of galactose toxicity in the rat on development of the ovary and testis. Amenorrhea with ovarian failure at an early age appears to be characteristic clinically of female galactosemia. If successful, our attempts to demonstrate relationships

between gonadal development and toxicity from galactose or its metabolites would provide important new areas for investigation of toxic substances affecting germ cells.

In the cytogenetic area, a new chromosomal rearrangement involving the X chromosome has been identified (pericentric X inversion). Furthermore, a unique individual with a 45, X karyotype and the presence of testicular tissue has been identified and studies on the relationship between his pattern of differentiation and the H-Y antigen are under investigation.

A number of studies are being undertaken in the areas of somatic cell genetics. We previously demonstrated that human chromosomes can be enclosed in lipid envelopes and transferred into HGPRT deficient mouse A9 cells in a higher frequency of incorporation than previously reported. Large chromosomal fragments can be integrated into the recipient genome and such integration appears to have some site specificity. We are now using liposome enclosed DNA, chromatin, and chromosomes in studies designed to test whether irreversible alterations in DNA are characteristic of the process of X chromosome inactivation. An improved method for encapsulating DNA into liposomes has been developed in the course of these studies.

Because of the importance of prenatal diagnosis in many genetic disorders, we have been interested in improving techniques for growing amniotic cells in culture. A controlled double blind study has now demonstrated that fibroblast growth factor from sheep brain can significantly stimulate the growth of human amniotic cell lines. Other studies involving prenatal diagnosis have included the demonstration that the prenatal diagnosis of the adrenogenital syndrome can be achieved by measuring 17 hydroxyprogesterone and androstenedione levels in amniotic fluid.

Significance to Biomedical Research and Programs of the Institute: Cystinosis, homocystinuria, phenylketonuria, and the inborn errors of glutathione metabolism all present clinical pictures of varying degrees of severity, in some cases lethal. These and other heritable disorders studied in our laboratory require better methods of diagnosis, treatment and prevention than are currently available, and our efforts in these directions are essential for the goals of this Institute. In addition, it has been frequently demonstrated that the study of genetic variants in man is a powerful tool for advancing our understanding of normal and abnormal biochemistry. Our studies on glutathione metabolism illustrate that point. Furthermore, the extension of our work from rare genetic disorders of glutathione metabolism to an extremely common one like glucose-6-phosphate dehydrogenase deficiency demonstrates how the study of rare model defects may open the way to understanding of common disorders with substantial public health significance. An interest in genetic diseases in general should include an interest in more fundamental aspects of human genetics. Our studies related to mechanisms of cell growth and gene integration and inactivation are consonant with this orientation.

Proposed Course of Project: Future efforts will include: 1) the continuing study of compounds which may be of potential therapeutic benefit in cystinosis and other heritable disorders; 2) further investigation of the mechanisms of lysosomal metabolism of cystine and related compounds in cystinosis; 3) continued investigation of the biochemical abnormalities in G6PD deficiency; 4) continued studies of the enzymes responsible for the synthesis and degradation of glutathione, and their involvement in disorders of its metabolism; 5) the development of an in vivo radiochemical assay for phenylalanine hydroxylase activity in patients with PKU and other abnormalities of phenylalanine metabolism; 6) investigation of additional inborn errors of metabolism, especially adrenal leukodystrophy and galactosemia; 7) using gene transfer technology with the hope of understanding and developing approaches to gene therapy of genetic defects.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00132-04 NPMB

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Differentiation of Intestinal Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

N. Kretchmer	Head, DGN Section	NPMB, NICHD

Other Investigators:

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K. Berry	Chemist	NPMB, NICHD
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COOPERATING UNITS (if any)
W. Rutter, Dept. Molecular Biophys., UCSF; J. Bernstein, William Beaumont Hospital, Royal Oak, Michigan; G. Semenza, Lab. Biochem. Tech. Inst., Zurich, Switzerland; J. Roth, NIAMDD, NIH

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Section on Developmental Gastroenterology and Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Section on Developmental Gastroenterology and Nutrition was established to study (1) intestinal epithelial cell migration and differentiation and (2) the nutritional modifications of gene activity and expression in developing and adult mammals. With respect to epithelial cell migration, our efforts have been focused on the establishment of a primary cell culture system which will allow us to define those cell surface components which are responsible for cell attachment to the basement membrane. In addition, an intestinal organ culture system is being developed as an in vitro model of cellular locomotion along the crypt-villus axis, so that the roles of protein synthesis and hormonal influences on intestinal cell migration can be investigated. We are also continuing our ongoing research into the control of sucrase/isomaltase synthesis and the mode of incorporation of the complex into the intestinal brush-border membrane. Primary cultures of rat pancreatic exocrine cells are being established to elucidate the role of nutrition in the modulation of gene activity and expression. Initially, studies will focus on the mechanism of adaptation of rat amylase in isolated cells and intact pancreas to carbohydrate in the media or the diet.

Project Description:

Objectives: Fundamental to the biology of cells and the organization of multicellular systems is the ability of cells to adhere to and migrate along an extracellular matrix. As a model for the study of these related phenomena, we have chosen the intestinal epithelium, where an orderly sequence of cell division and differentiation, concomitant with cellular migration from the crypt to villus tip, results in renewal of the epithelium within two to three days (in the rat). A major effort is being made to (1) identify the cell surface component(s) which anchor the epithelium to its basement membrane, (2) discover the mode of association of this attachment component with both the cytoskeletal structures and the basement membrane and (3) analyze the roles of protein synthesis, cellular metabolism and humoral agents in controlling epithelial locomotion. Furthermore, the regulation of the expression of the enzyme complex, sucrase/isomaltase and its incorporation into the brush-border-membrane remains a major concern of this laboratory. In our attempt to understand the role of nutrition in cellular metabolism, we are investigating the mechanism of adaptation of amylase in primary cultures of pancreatic exocrine cells derived from rats at various stages of development. This adaptation provides a model within which to study nutritional modifications of gene activity and expression throughout the development of the organism. Also, the evolution of this adaptation can be examined by comparing its mechanisms in species that differ in evolutionary age and complexity.

Methods Employed: The principal methods employed are: (1) cell and organ culture techniques, (2) cell dissociation techniques, (3) bright field, phase contrast, immunofluorescent and electron microscopy, (4) column chromatography (affinity, ion exchange, and gel filtration), (5) polyacrylamide slab and disc gel electrophoresis, (6) immunoelectrophoresis, (7) centrifugation and ultracentrifugation, (8) spectrophotometric, fluorometric and radiochemical assays (9) autoradiography, (10) amino acid analysis, and (11) cDNA hybridization.

Major Findings: We are currently using hyaluronidase in a two step procedure to obtain separate populations of villus cells and crypt cells, from either rat or rabbit intestine. Villus cells have been maintained in culture for up to 96 hours on a variety of substrates,, including collagen Type I gels, urea denatured Type I films, native Type I films, collagen Type IV films, laminin films and fibronectin films. With the exception of collagen Type I gels, only about 10% of the initial number of epithelial cells plated onto the various substrates were attached after 24 hours in culture. On collagen Type I gels 20 to 40% of the initial plating was still attached after 48 hours. The measurement of cell attachment over a short time period (1 to 2 hours) on each of the different substrates has revealed: (1) serum independence and/or inhibition of attachment, depending on the substrate; (2) in the absence of serum rat, cells attached to substrate in the decreasing order: Type IV film, Type I gel, laminin, fibronectin, Type I film and tissue culture plastic. In addition, we have purified intestinal basement membrane and found an increase in cell attachment with an increase in basement membrane concentration.

The enzyme complex, sucrase/isomaltase, has little or no catalytic expression in the crypt; however, as the cells migrate along the villus, this enzyme becomes progressively more active up to the mid villus region, followed by a moderate decline toward the tip of the villus. This enzyme has been associated with 6 nm knobs observed by negative staining with the electron microscope as protrusions of the brush-border membrane. We have found that the density of these knobs is nearly constant per unit membrane on cell surfaces isolated either from crypt cells or from differentiated cells obtained from either the base, mid, or tip regions of the villus.

Whereas most mammalian intestinal cells possess the complex sucrase/isomaltase, intestinal cells from sea lions, family Pinnipedia, only possess the isomaltase catalytic activity. This enzyme has been purified to homogeneity and evidence has been obtained which suggest that this single protein is a polypeptide chain that contains two active sites.

Rat amylases have been purified to homogeneity by a novel, one-step affinity chromatographic procedure developed in this laboratory. This procedure uses an α-glucohydrolase inhibitor (proprietary name: Bay G5421) coupled to AH-sepharose, as an affinity ligand, and allows for the rapid and highly efficient purification of both pancreatic and salivary amylase.

Rat pancreatic exocrine cells have been separated and cultured for 2-3 weeks. These cells maintain their differentiated morphology, but do not appear to grow in culture. The cultured exocrine cells secrete α-amylase activity into the media and maintain a relatively stable, cellular pool of amylase activity.

Significance to Biomedical Research and the Program of the Institute: Definition of the processes involved in differentiation and cell migration may aid in the understanding of malabsorption states such as chronic diarrhea of infancy, short bowel syndrome, coeliac disease and other pathologic states where regeneration of the intestinal mucosa is a major concern. In addition, the understanding of the interaction of cells with the extracellular matrix may add to our knowledge of embryogenesis, organogenesis, tumor metastases, etc.

Changes in the nutritional environment during development and growth may modify differentiation and maturation of cellular processes. To understand this phenomenon better, we need to clarify the process of interaction between nutrients and gene expression. The development of a model system to study the significance of nutritional factors in gene expression is in progress.

Proposed Course of the Project: We are continuing our efforts to define the specific interaction of intestinal epithelial cells with various substrates using primary cell cultures. We are also using an intestinal organ culture model to investigate the roles of protein synthesis, energy metabolism, and humoral agents in the regulation of cell migration. Furthermore, we are investigating the relationship of the sucrase/isomaltase complex with the 6 nm knobs using cultures of crypt cells exposed to cortisone and or insulin,

since these hormones are known to induce sucrase/isomaltase activity.

Antibodies against α-amylase are being prepared to be used in the development of ELISA and immunoadsorbent techniques for the detection of the enzyme. Cultured pancreatic exocrine cells will be further characterized by examining β-islet cell contamination and maintenance of differentiated biochemical functions in vitro. The proposed roles of dexamethasone and insulin in the regulation of the adaptation of α-amylase will be investigated in cultured exocrine cells. Finally, the development of this regulation will be studied in vitro in exocrine cells isolated from embryonic rats at various stages of development.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00133-03 NPMB

PERIOD COVERED: October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study of Type II Glycogenosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. B. Sidbury, M.D.	Scientific Director	NPMB, NICHD
	Paz Abastillas, M.D.	Visiting Fellow	NPMB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION

INSTITUTE AND LOCATION
National Institute of Child Health and Human Development, Bldg. 10, Rm. 8D-45

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There are now several forms of variants of myopathy associated with absent or deficient lysosomal α glucosidase. The classical Pompe's disease, type II glycogenosis has an absence of the enzyme in all tissues except the kidney, and the heart is prominently involved. A juvenile form, i.e., later onset, presents simulating muscular dystrophy. Here, too, the enzyme is absent, but the heart is not clinically involved. This constellation can be seen in older children and adults where the myopathy is predominant. There is also a form of myopathy where the enzyme is markedly lowered and the Km 1/2 the normal. We are trying to isolate the enzyme from the urine to perform genetic studies and in order to use this source of enzyme to better characterize the enzyme.

Project Description ZO1 HD 00133-03 NPMB

Objectives:

1. To study the properties of myopathies associated with increased glycogen and reduced lysosomal α glucosidase.
2. To further characterize the enzyme.
3. To produce antibodies to placental lysosomal glucosidase and renal cortical glucosidase.
4. To utilize the antibody to placental lysosomal glucosidase to study the role of lysosomal glucosidase in the normal metabolism of glycogen in fibroblast.
5. To develop antibodies to renal glucosidase to use in separating the renal from liver-muscle glucosidase.

Methods Employed:
Tissues are assayed for lysosomal α glucosidase using the umbilliferone pyranoside, following the fluorescence. The pH 4 and pH 6.5 activities are determined. The assay is run at different substrate levels and using different inhibitors and stimulators. The urine is assayed at pH 4 and pH 6.5. The urine is concentrated and run over a G-100 Sephadex column to separate the pH 4 and 6.5 activities. An improved method for separating these two activities is needed.

Major Findings:
We have found 2 patients with low, not absent, lysosomal α glucosidase and moderate glycogen accumulation. The patients presented clinically with myopathy. The enzyme was shown to have a V_{max} only 50% of that found in normal muscle. Clinically, there is the suggestion that the condition may be dominant, i.e., one mother-daughter involvement. Surprisingly, the glucosidase of the fibroblasts showed low activity but not an altered V_{max}. There are several possible interpretations of this.

It now becomes obvious that the renal isozyme has distinct properties. Antibodies to each isozyme have been described. We have found that the renal isozyme is precipitated by alcohol, whereas the liver-muscle isozyme is not. This has been particularly helpful with urine samples.

Proposed Course:
We proposed to attempt to use antibody to placental glucosidase to study the role of the lysosomal glucosidase in normal cells. We will develop antibodies to renal glucosidase in order to be able to quantitate the renal vs. liver-muscle glucosidase in urine.

Publications:
None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00134-03 NPMB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study of Obesity in Children with Prader-Willi Syndrome and Exogenous Origin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. B. Sidbury, M.D.	Scientific Director	NPMB, NICHD
	Paz Abastillas, M.D.	Visiting Fellow	NPMB, NICHD
	Carol Bruner, Ph.D.	Guest Worker	NPMB, NICHD
	Ann McNemar, R.N.	Nurse	NPMB, NICHD
	Lawrence Tamarkin, Ph.D.	Staff Fellow	NPMB, NICHD

COOPERATING UNITS (if any)

Clinical Center Nutrition Department and Physiotherapy Department
Nursing Department and Social Service

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION

INSTITUTE AND LOCATION
National Institute of Child Health and Human Development, Bldg. 10, Rm. 8D-45

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Base-line glucose tolerance tests with insulin, prolactin release with thorazine, and the circadian rhythm of cortisol, GH, prolactin, LH, FSH are obtained. A low carbohydrate, low calories diet is initiated for weight reduction. Follow up studies of glucose tolerance and circadian patterns of the several hormones are repeated periodically during the course of weight reduction. The study will attempt to detect evidence of altered hormonal regulation in obesity with emphasis on the CNS role, including certain brain peptides.

PHS-6040

Project Description: Z01 HD 00134-03 NPMB

Objectives:

1. To determine insulin responses under different conditions in patients with Prader-Willi Snydrome (P.W.S.) as compared with exogenous obesity (EO).
2. To compare prolactin release after thorazine injection in the P.W.S. vs. EO and the circadian pattern.
3. To compare circadian pattern of hGH, LH, FSH, cortisol in P.W.S. vs. EO.
4. To determine circadian pattern of melatonin in obese state and after weight reduction.
5. To determine the effect of weight loss on these parameters.

Methods Employed:

Patients with P.W.S. and EO who are at least 50% above their ideal weight for height are studied. Their "normal" caloric intake is estimated. A glucose tolerance test with determination of glucose and insulin is performed. The rise in prolactin following an injection of 0.7 mgm of thorazine/Kg ideal body weight is ascertained over 2 hours. The circadian pattern of LH, FSH, hGH, cortisol, prolactin and melatonin is determined every 2 hours over a 24-hour period. The assays are performed by radioimmunoassay. Comparisons will be made between the response in P.W.S., EO and normal weight individuals. The diet prescribed is low in carbohydrates and calories. Exercise is prescribed.

Major Findings:

Insulin response to glucose and a normal meal is exaggerated and prolonged. 80% of obese patients fail to show a prolactin release with thorazine administration. The very small number of patients whose circadian pattern for prolactin has been determined show a normal pattern despite the failure to respond to thorazine. The only patient who has failed to show a circadian rhythm for cortisol is a single post-pubescent P.W.S. All prepubescent P.W.S. patients and EO patients have a normal pattern. The circadian pattern of P.W.S. patients over 12 years of age has varied from exaggerated levels to no circadian rhythm. Obese children tend to have slightly higher levels than normal. GH and prolactin response has returned to normal after weight reduction. The response to the weight reduction program in both groups is similar.

Significance to Biomedical Research and the Program of the Institute:

Obesity is the major nutritional problem in the U.S. today. No abberations in metabolism or regulation have been discerned and confirmed except increased insulin levels, increased insulin resistance, and decreased insulin binding to cells and blunted hGH response to challenge. The present program is the beginning of a search for other abnormalities of regulation.

Proposed Course:

We plan to test the effect of Naloxone in appetite, behavior and the peptide response in the blood. Further studies will be done to elucidate the interaction of food substances with brain peptides and the sequential relationships of the peptides.

Project Description: Z01 HD 00134-03 NPMB

Publications:
None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00135-03 NPMB

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Developmental Endocrinology and Neuroendocrinology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Barry B. Bercu
Gary Francis, Clinical Associate, NPMB, NICHD

Other Investigators: George Chrousos, Clinical Associate, NPMB, NICHD
Merrily Poth, Clinical Associate, NPMB, NICHD
Teri Brown, Biology Laboratory Technician
Joseph Ober, Student Scientist
Hari Brown, Student Scientist

COOPERATING UNITS (if any)

SEE ATTACHED SHEET

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the development of the hypothalamic-pituitary-testicular axis. We have developed bovine and rat Sertoli cell culture systems. We have examined autoregulation of FSH response in rat Sertoli cells and FSH stimulation of ornithine decarboxylase activity in the bovine cells as well as other similarities and differences between the two culture systems.

We have used luteinizing-hormone releasing hormone antiserum to further study the hypothalamic-pituitary-testicular axis. We have studied its effect on the development of the secretion of gonadotropins, androgen binding protein, testosterone and the number of luteinizing hormone receptors.

We have developed an animal model to study the hypothalamic-pituitary axis in primates (rhesus monkeys). We have studied the effects of cranial radiation as well as neuropharmacologic drugs and hormones on the secretion of pituitary hormones.

Further studies on pituitary resistance to thyroid hormone in cystinosis have been completed. Both in vivo and in vitro studies are in progress in order to better define the defect.

PHS-6040

Cooperating units:

P. Donahoe, Massachusetts General Hospital

D. Poplack, A. Oliff, Pediatric Oncology, NCI

I.M.D. Jackson, Tufts - New England Medical Center

R. Johnsonbaugh, National Naval Medical Hospital

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J. Schulman, NICHD

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T. Triche, NCI

J. Butler, NICHD

A. Mukherjee, PRB, NICHD

E. Quinlan and P. Kornblith, NINCDS

J. Schwade, NCI

G. Gunsalus, W. Bardin, Rockefeller University

Project Descriptions:

Objectives: The goals of the project are as follows: (1) to explore the development of the hypothalamic-pituitary-gonad axis, (2) to elucidate the biochemistry and function of the bovine rat Sertoli cells, (3) to develop a human model for studying Sertoli cell function, (4) to explore intersex problems with relationship to Mullerian Inhibiting Substance and inhibin, both are hormones which are made by the Sertoli cell, (5) to utilize our neuroendocrine primate model (rhesus monkey) to study the effect of cranial radiation and various drugs on hypothalamic-pituitary secretion, (6) to further study pituitary resistance to thyroid hormone in cystinosis and other metabolic disorders, (7) to evaluate metabolic effects of hGH in growth hormone deficient patients, (8) to further study neuroendocrine abnormalities in patients who have received cranial irradiation.

Methods Employed: The principal methods employed are: 1) cell culture techniques, 2) radioimmunoassay of hormones, 3) radioreceptor assays, 4) hormone bioassays, 5) thin layer chromatography, 6) polyacrylamide gel electrophoresis, 7) radioisotopic measures using tritium, C_{14}, I-125, 8) immunization techniques, active and passive, 9) animal surgery, rat and primate, 10) animal cannulation techniques, 11) measurement of cyclic nucleotides, 12) viral cell transformation, 13) irradiation techniques.

Major Findings: Various functions of rat and bovine Sertoli cell systems have been compared. We have measured several FSH stimulated functions including: cyclic AMP and conversion of testosterone to estradiol. FSH stimulates ornithine decarboxylase activity in bovine cells. We have demonstrated autoregulation of FSH response in rat Sertoli cells and we have also studied FSH receptors in this tissue culture system.

Using passive immunization of young rats with LHRH antisera we have further studied the development of the hypothalamic-pituitary-testicular axis. Gonadotropin, androgen binding protein, testosterone secretion and hCG/LH receptors were measured. The male hypothalamic-pituitary system appears more sensitive than the female system during early development.

Exogenous LHRH administration in rats causes uterine inertia in late pregnancy. The estradiol/progesterone ratio is reversed and oxytocin and nuclear estradial receptors are altered.

A useful animal model to study neuroendocrine function in primates was developed. The following studies are partially complete: effect of cranial irradiation and various drugs including somatostatin, melatonin, bromoergocriptine, α and β stimulants and B blockers, and serotoninergic antagonists.

Patients with cystinosis appear to have pituitary resistance to thyroid hormones.

We have demonstrated somatostatin in primate cerebral spinal fluid.

Project Descriptions:

Objectives: The goals of the project are as follows: (1) to explore the development of the hypothalamic-pituitary-gonad axis, (2) to elucidate the biochemistry and function of the bovine rat Sertoli cells, (3) to develop a human model for studying Sertoli cell function, (4) to explore intersex problems with relationship to Mullerian Inhibiting Substance and inhibin, both are hormones which are made by the Sertoli cell, (5) to utilize our neuroendocrine primate model (rhesus monkey) to study the effect of cranial radiation and various drugs on hypothalamic-pituitary secretion, (6) to further study pituitary resistance to thyroid hormone in cystinosis and other metabolic disorders, (7) to evaluate metabolic effects of hGH in growth hormone deficient patients, (8) to further study neuroendocrine abnormalities in patients who have received cranial irradiation.

Methods Employed: The principal methods employed are: 1) cell culture techniques, 2) radioimmunoassay of hormones, 3) radioreceptor assays, 4) hormone bioassays, 5) thin layer chromatography, 6) polyacrylamide gel electrophoresis, 7) radioisotopic measures using tritium, C_{14}, I-125, 8) immunization techniques, active and passive, 9) animal surgery, rat and primate, 10) animal cannulation techniques, 11) measurement of cyclic nucleotides, 12) viral cell transformation, 13) irradiation techniques.

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Patients with cystinosis appear to have pituitary resistance to thyroid hormones.

We have demonstrated somatostatin in primate cerebral spinal fluid.

Significance to Biomedical Research and Programs of the Institute: The focus of our laboratory is to develop better methods for diagnosis and treatment of disorders relating to developmental endocrinology and neuroendocrinology.

This includes intersex problems, especially those that require the correct diagnosis and treatment in the newborn period. In addition, we have studied neuroendocrine complications of cranial radiation, a technique commonly used in pediatric oncology. Using our primate model, we should be better able to understand the effects of drugs and hormones on hypothalamic-pituitary function. Our efforts in these directions are directly related to the goals of this institute because of their applications for advancing our understanding of child development.

Proposed Course of Project: Future efforts will include: 1) the continuing study of the development of the hypothalamic-pituitary-gonad axis, 2) further investigation of Sertoli cells and their functions, 3) continued studies of the abnormalities of the hypothalamic-pituitary axis of humans and monkeys, 5) further studies on Mullerian Inhibiting Substance and inhibin, 6) investigation of intersex problems, 7) continued studies of the hypothalamic-pituitary-thyroid and gonadal axis in cystinosis.

Publications:

1. Bercu, B.B., Morikawa, Y., Jackson, I.M.D., and Donahoe, P.K.: Inhibition of Mullerian Inhibiting Substance secretion by FSH. Ped. Res. 13: 246-249, 1979.

2. Beitins, I.Z., Dufau, M.L., O'Loughlin, K., Bercu, B.B., McArthur, J.W., Crawford, J.D., and Catt, K.J.: Comparison of biological and immunological activities of serum LH in normal women during LHRH infusion and patients with Turner's Syndrome treated with estrogens. In press.

3. Bercu, B.B., Jackson, I.M.D., and Reichlin, S.: Delayed puberty after immunological blockade of endogenous LHRH in female rats. Proc. Soc. Biol. Med. 160: 123-125, 1979.

4. Bercu, B.B. and Donahoe, P.K.: Mullerian Inhibiting Substance in abnormalities of sexual differentiation. OB GYN Series, In press.

5. Bercu, B.B. and Schulman, J.D.: Genetics of abnormalities of sexual differentiation and of female reproductive failure. OB GYN Survey 35: 1-11, 1980.

6. Bercu, B.B., Donahoe, P.K.D., and Schulman, J.D.: Developmental abnormalities of the mullerian duct. OB GYN Series, In press.

7. Oliff, A., Bode, U., Bercu, B.B., DiChiro, G., Graves, V., and Poplack, D.G.: Hypothalamic-pituitary dysfunction following CNS prophylaxis in acute lymphocytic leukemia: correlation with CT scan abnormalities. Med. and Ped. Onc. 7: 141-151, 1979.

8. Bercu, B.B., and Jackson, I.M.D.: Response of adult male rats to LH-RH after neonatal immunization with antiserum to LH-RH. J. of Reprod. and Fert. 59: 1980.

9. Bercu, B.B., Morikawa, Y., and Donahoe, P.K.: Control of mullerian inhibiting substance secretion. Clinics in Andrology, In press.

10. Bercu, B.B. and Donahoe, P.K.: LH-RH analogs and the undescended testis. In Fonkalasrud, E. (Ed): "The Undescended Testis". Year Book Medical Publisher, In press.

11. Bode, U., Oliff, A., Bercu, B.B., DiChiro, G., Glaubiger, D., and Poplack, D.: Absence of CT brain scan abnormalities with less intensive CNS prophylaxis. Amer. J. Pediatr. Hemat. and Oncology 2: 21-24, 1980.

12. Bercu, B.B., Hyashi, A., Poth, M., Soloff, M.S., Alexandrova, M., and Donahoe, P.K.: LH-RH induced delay of parturition. Endo. 1980, In press.

13. Oliff, A., Bode, U., Bercu, B.B., DiChiro, G., Graves, V., Pizzo, P., Glaubiger, D., Glatstein, E., and Poplack, D.: Abnormally low growth hormone (GH) responses to insulin induced hypoglycemia following central nervous system (CNS) prophylaxis: correlation with abnormal computerized tomographic (CT) brain scans. Am. Soc. of Clin. Oncology, 1979.

14. Bercu, B.B., Hyashi, A., Poth, M., Soloff, M.S., Alexandrova, M., and Donahoe, P.K.: LH-RH induced delay of parturition. Sixth Int'l Cong. of Endocrinology, Melbourne, Australia, Feb. 1980.

15. Francis, G. and Bercu, B.B.: Autoregulation of FSH response in rat sertoli cells (RSC). Soc. for Ped. Res., San Antonio, April 1980.

16. Chrousos, G.P., Poplack, D., Oliff, A., Schwade, J.G., and Bercu. B.B.: Acute effects of cranial irradiation on regulation of neuroendocrine functions in adult male rhesus monkeys. Soc. for Ped. Res., San Antonio, April 1980.

17. Francis, G., Triche, T., and Bercu, B.B.: FSH stimulation of bovine sertoli cell ornithine decarboxylase activity. Endocrinology Society Meeting, Washington, D.C., June 1980.

18. Bercu, B.B., Hyashi, A., Poth, M., Soloff, M.S., Alexandrova, M., and Donahoe, P.K.: LH-RH induced delay of parturition. Endocrinology Society Meeting, Washington, D.C., June 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00401-02 NPMB

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Disorders of Carbohydrate Metabolism in Infancy and Childhood

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigators: Marvin Cornblath

Other Investigators: George Chrousos
William Nelson III
Anthony Adams

COOPERATING UNITS (if any)

Jesse Roth and Robert Dons, Diabetes Branch, NIAMDD
Avinoam Kowarski, Endocrine Division, Dept. of Pediatrics, Johns Hopkins

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our major goal is the elucidation of the pathophysiology in infants and children with disorders of carbohydrate metabolism, such as hypoglycemia, Glycogen Storage Disease, and Diabetes Mellitus. This should lead to understanding the physiology of carbohydrate regulation and the developmental role of hormones and their receptors in both health and disease. Diet and other treatment modalities will be investigated as well, e.g. long-acting zinc glucagon has been found effective in 1 child with hypoglycemia.

Additional studies of erythrocyte insulin receptors have verified their increased numbers in patients with symptomatic hypoglycemia, and following the introduction of nocturnal continuous nasogastric feeding in GSD I. Diazoxide® appears to reduce specific I^{131} insulin binding to red cells. Studies of glycosylated Hbs show significant elevations of the negatively charged minor hemoglobins ($HbAI_{a+b}$) in patients with GSD Type I and fructose-diphosphatase deficiency. $HbAI_c$ was not changed.

Continuous glucose monitoring will be started to better evaluate changes with diet, continuous nasogastric feeds, and tolerance tests in children and adults.

PHS-6040

Other Collaborations:

Dr. Robert Schwartz, Brown University

Dr. Noel MacLaren, University of Florida

Dr. Avner Notkin, NDR, NIH

Dr. Judith McLaughlin, University of Maryland

Dr. Avinoam A. Kowarski, Johns Hopkins

Dr. Herbert Schwartz, Stanford University

Project Description

Objectives:

The goals of the project are as follows: (1) to elucidate the pathophysiologic mechanisms involved in various disorders of carbohydrate metabolism in infants and children. (2) To evaluate the currently used diet and therapeutic regimens in the above disorders and modify them as indicated. (3) To study the relationship of hormone effectors, their receptor sites and other parameters relevant to carbohydrate metabolism such as concentrations in blood of glucose, lactate, pyruvate, free fatty acids, ketones, and amino acids. (4) To assess the overall growth and development of these patients by following their growth patterns, the maturation, function and response of their neuroendocrine and peripheral endocrine systems as well as the development of their neurological and psychological behavior. (5) To establish and study appropriate animal models (primate, rodent, etc.) for each of these areas.

Methods Employed:

The research methodology employed can be separated in (a) clinical and (b) laboratory.

(a) The patients are studied clinically on the 9W pediatric ward of NICHD. Complete physical evaluations, neurological examinations and psychological testings are performed. Diagnostic tests available routinely at the Clinical Center will include EEG, diagnostic x-rays, CAT scans, clinical pathology tests etc. as indicated.

(b) Laboratory investigative tests are performed to study three distinct, but related areas: (1) Insulin receptor sites (number of receptors and affinity to insulin) on circulating erythrocytes, measured by a radioreceptor assay, (2) Circulating insulin, growth hormone cortisol, T_4, and glucagon measured by radioimmunoassay and (3) biochemical determination of substrates and metabolites that either reflect or influence carbohydrate metabolism by micro-automated fluorometric techniques. Liver enzyme studies will also be done as indicated. A constant glucose monitor developed by Dr. Kowarski will be available to study patients with carbohydrate disorders in a more precise and effective manner.

Major Findings:

(1) Projects and studies - Glycosylated hemoglobin functions have been measured in hypoglycemic syndromes and in GSD, Types I, Ia, III and IX in collaboration with Dr. Robert Schwartz and Dr. Herbert Schwartz.

Several minor hemoglobins were measured by high performance liquid chromatography in children with a variety of disorders associated with hypoglycemia. No abnormalities of Hb AI_c were found in subjects with glucose-6-phosphate deficiency or fructose-1,6-diphosphatase deficiency. However, significant elevations of the negatively charged minor hemoglobins (Hb AI_{a+b}) were found in all patients with glucose-6-phosphatase or fructose-1,6-diphosphatase deficiency. Chemical characterization of the hemoglobin adduct should contribute to a further understanding of the metabolic alterations in these disorders and may contribute to evaluating modalities of treatment.

(2) An unusual family with onset of insulin dependent diabetes mellitus in 2 of 4 siblings within a 3 week-period has prompted a study of viral antibodies, HLA typing, toxicological studies, etc. of the entire family. The 13 month old and 6 year old girls became diabetic. To date, only the younger child has islet antibodies whereas everyone in the family has a high anti-nuclear antibody titer in their plasmas. Other results are pending.

(3) One of identical twin adults had a circulating anti-alpha cell antibody which was revealed in a family study of insulin dependent diabetics (5 from these 2 sibships) by Dr. MacLaren. Arginine tolerance tests were performed in both twins to determine glycagon responses. Data on insulin, HGH, and glucagon are pending. Both showed a normal glucose response to arginine.

(4) In studies of familial nesidoblastosis one patient was found to have a relative glucagon deficiency in response to arginine and to induced hypoglycemia. Dramatic improvement occurred with twice daily injections of long-acting zinc glucagon. The usefulness of this hormone will be explored in other patients with recurrent or severe hypoglycemia.

(5) The long-term follow-up, management, and erythrocyte insulin receptor data summarizing 9 patients with hyperinsulinemic hypoglycemia since infancy will be prepared for publication.

Significance to Biomedical Research and Programs of the Institute:

The focus of our research is to elucidate the pathophysiology of disorders of carbohydrate metabolism in infancy and childhood and develop better methods for diagnosis and therapy. Through these efforts the development of the normal child will be better understood. Both objectives are in concert with the goals of the Institute.

Proposed Course of Project:

Future efforts will include: (1) the continuing study of the pathophysiology of carbohydrate metabolism disorders in infancy and childhood. (2) a detailed study of the ontogeny of insulin receptors and the interrelationships of insulin and insulin receptors, throughout early development in animal models and man. (3) evaluation of the efficacy and effects of continuous nocturnal nasogastric feedings in children with Glycogen Storage Disease type I and its effects on the carbohydrate regulatory system. (4)

The effects of Diazoxide® in the treatment of hypoglycemic syndromes in infants and children and elucidation of its mechanism of action especially concerning receptor and postreceptor events. (5) Further study of nesidioblastosis which seems to be a familial disease. (6) The role and interrelationship of glucagon, somatostatin, insulin and growth hormone with insulin receptors and the overall change during growth and development. (7) Continuous glucose monitor evaluation of carbohydrate homeostasis in GSD, in persistent or recurrent hypoglycemia and in reactive hypoglycemia.

Publications:

1. Cornblath, M., Chrousos, G., and Adams, A.: Hormonal interactions and glucose homeostasis. Acta Paediatrica Belgica 32:231, 1979.

2. Chrousos, G.P., Wachslicht-Rodbard, H., Adams, A., Roth, J., and Cornblath, M.: I^{125}-Insulin binding to erythrocytes of hypoglycemic infants and children. Submitted for publication.

3. Chrousos, G.P., Wachricht-Rodbard, H., Adams, A., Roth, J. and M. Cornblath: Insulin binding to erythrocytes in infants with abnormlaities of glucose metabolism. Submitted for publication.

4. Zeller, W.P., Cornblath, M., Schwartz, H.C., and Schwartz, R.: Minor hemoglobins in disorders of carbohydrate metabolism. Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00402-02 NPMB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mass Spectrometry Facility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alfred L. Yergey	Chemist	NPMB, NICHD
OTHER:	Edward Seeliger	Clinical Associate	NPMB, NICHD
	Lawrence Tamarkin	Staff Fellow	NPMB, NICHD
	Nancy Vieira	Biologist	NPMD, NICHD

COOPERATING UNITS (if any)
J. Hansen, NPMB, NICHD; N. Gershfeld, LPB, NIAMDD; M. Friedman, U.S. Army; J. S. Sidbury, NPMB, NICHD; J. Ehrenkranz, DEB, NICHD; M. Lippman, DCI, NCI; B. Chabner, CT, NCI; S. Binkley, Temple University, PA.

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION

INSTITUTE AND LOCATION
National Institute of Child Health and Human Development, NIH, Bldg. 6, Rm. 140

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.50	3.50	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Quantitative analysis using solids inlet probe are being done in 4 areas: (1) Calcium isotope ratios by thermal ionization for studies of calcium kinetics; (2) Disaturated lecithins as an assay for a potential independent risk factor in atherosclerosis; (3) 13C labeled amino acids for use as tracers in metabolic studies; (4) Melatonin as a methodologically independent validation for melatonin RIA results. Multivariate statistical methods are being applied to large mass spectral data sets. Mathematical modeling studies of quadrupole mass spectrometers are being conducted.

The physiologic role and regulation of the pineal hormone melatonin is being studied in human subjects and animals. Plasma melatonin is being studied in obese children, in precociously pubescent children, and in women with breast cancer. Pineal melatonin is being measured in rodents to investigate the circadian regulation of this hormonal and its onogeny. Estrogen and progesterone receptors are being measured in hamster uteri to study the effect of acute melatonin treatment on these receptors. The inhibitory effect of melatonin on the induction of mammary tumors in dimethylbenzathracene treated rats is being studied. In vitro production of melatonin by a single chicken pineal gland is being studied.

PHS-6040

Mass Spectrometry Project Description Z01 HD 00 402-02 NPMB

Objectives: Develop mass spectrometric methods for analysis of substances for which satisfactory analyses do not presently exist, particularly the quantitative measurement of stable isotopically labeled materials.

Develop techniques for analysis of data produced in pyrolysis of solid materials within the mass spectrometer source volume.

Develop models to increase understanding of and to improve quadrupole mass filters.

Methods Employed: Quantitative analyses are performed by use of specific ion monitoring (SIM) of substances evolving from a heated mass spectrometer solids inlet probe. Using special purpose computers developed for this task, intensity data from particular ions are accumulated in storage registers; ratios of total ion intensities from different registers are obtained after evolutions are complete. Quantitation is obtained by using an internal standard that co-evolves with the analyate. The standard is either a chemical homolog or an isotopically labeled form of the analyate.

APL language as implemented on the PDP-10 is used for the multivariate statistical analysis of pyrolysis data sets. This language is selected because of the great convenience it offers for the manipulation of the large data sets produced in pyrolysis mass spectrometry.

Construction of functioning physical models as well as analytical and numerical solutions of the differential equations of motion that describe ion trajectories in multiple fields are used in the development of models for quadrupole mass filter improvement.

Major Findings: (1) Calcium isotope ratio measurements that are accurate to about 1% of natural abundance and which are reproducible to about 1% have been used to measure tracer quantities of the stable isotopes ^{44}Ca and ^{48}Ca in urine specimens. The methodology has been applied to the study of calcium metabolism in human subjects. The method has been extended to permit the measurement of the low abundance (.0033% of natural abundance) ^{46}Ca isotope. (2) Analysis of disaturated lecithins by ammonia chemical ionization has been carried out on three compounds thought to be of significance in atherosclerosis. Quantitation accuracy is about $\pm$ 5% with precisions of about the same level. This analysis is the first mass spectrometric quantitative analysis of lipids. (3) Preliminary results of the melatonin analysis indicates that the method being developed in this laboratory is significantly easier to use than the current standard mass spectrometric analysis, yet it yields results of satisfactory sensitivity for corroboration of RIA analyses. The preliminary sensitivity studies show that extraction of 6 ml of serum known to have 40 pg/ml gives signal to noise levels greater than 10:1. (4) All of the essential amino acids as underivitized molecules yield protonated molecular ions from isobutane chemical ionization and only leucine/isoleucine cannot be differentiated by this approach. Extraction of amino acids added to plasma over a 10-fold concentration range has yielded linear response curves for all of those compounds studied to date. (5) Programs using APL have been developed to take mass spectral files into the PDP-10, condition them for analysis and manipulate them so that characteristic

patterns in the evolution of thermal degradation products can be recognized. (6) A working mechanical model of an analog of an ion oscillating in one plane of a quadrupole mass filter has been constructed. Measurements made from motion pictures of the model represent the first experimental determination of the characteristic frequency of such oscillations. Differential equations of motion for ions in fields with more than 4 symmetrical potential surfaces have been determined.

Proposed Course: (1) Mass spectrometry measurements will continue in the 4 areas described while time will be provided for analysis of particular samples as the need arises. (2) Programming will be developed to effect a rapid transfer of large data sets between the mass spectrometer data system and the PDP-10. (3) Numerical integration of the higher order multipole field equations will be undertaken in order to evaluate those geometries for improved mass filter devices.

Publications:

1. A. L. Yergey, N.E. Vieira, and J. W. Hansen, "Isotope Ratio Measurements of Urinary Calcium with a Thermal Ionization Probe in a Quadrupole Mass Filter," Anal. Chem., September 1980.

2. A. L. Yergey, "Report on the Workshop on Analysis by Solid Probe Held at the 27th Annual Conference of the American Society for Mass Spectrometry," Biomed Mass. Spect.

3. A. L. Yergey, M. H. Kody, N. L. Gershfeld, "Quantitative Analysis of Disaturate Lecithins by NH_3-with A Solid Probe," Proceedings of the 28th Annual Conference on Mass Spectrometry and Allied Topics, New York, N. Y., May 25-30, 1980.

4. A. L. Yergey, M. H. Friedman, J. E. Campana, L. Kelner and E. H. Seeliger, "The Inverted Pendulum as a Model of the Quadrupole Mass Filter," Proceedings of the 28th Annual Conference on Mass Spectrometry and Allied Topics, New York, N. Y., May 25-30, 1980.

5. M. H. Friedman, J. E. Campana, A. L. Yergey, L. Kelner, "Is There A Better Biometry than a Quadrupole?" Proceedings of the 28th Annual Conference on Mass Spectrometry and Allied Topics, New York, N. Y., May 25-30, 1980.

6. E. H. Seeliger and A. L. Yergey, "Identification and Quantiation of Amino Acids Using Solids Probe Chemical Ionization Mass Spectrometry," Ped. Res. 14, 581 (1980).

7. A. L. Yergey, N. E. Vieira, J. W. Hansen, "Calcium Isotope Ratios by A Thermal Ionization Probe in a Quadrupole Mass Spectrometer," Ped. Res. 14, 584 (1980).

Melatonin Project Description

Objectives: To understand the role of the melatonin rhythm in health and disease.

Methods Employed: The techniques employed are: (1) radioimmunoassay for melatonin and high performance liquid chromatography for validation of the assay; (2) quantitative mass spectrometry of melatonin for corroboration of the radioimmunoassay; (3) radioreceptor assays for estrogen and progesterone receptors to monitor the effect of exogenous melatonin.

Major Findings: In rodents: (1) The pineal melatonin rhythm in the Syrian hamster is truly circadian and may be regulated by two oscillators (biological clocks). (2) The pineal melatonin rhythm develops at a similar rate in seasonal and non-seasonal rodent species. (3) Injection of melatonin in prepubertal Syrian hamsters suppresses estrogen and progesterone receptors in the uterus. (4) Daily injection of melatonin for 3 months inhibits mammary tumor development in DMBA treated rats. Those tumor-free animals injected with melatonin had lower levels of plasma prolactin than vehicle-injected controls.

In vitro: (1) Chicken pineal glands cultured up to 5 days exhibit a daily rhythm in media melatonin provided the gland is exposed to a light-dark cycle; placing the gland in constant dark results in one peak followed by basal levels thereafter. (2) A one day culture of rat pup pineal glands also exhibit one cycle of the daily rhythm in media melatonin.

In human subjects: (1) Patients with true isosexual precocious puberty have a daily rhythm in plasma melatonin that is not different from control subjects. (2) Patients with Prader-Willi syndrome may have daily patterns of plasma melatonin that are different from exogenous obesity subjects. (3) Three of 5 women with nonmetastatic breast cancer did not have a nocturnal rise in plasma melatonin.

Proposed Course: In rodents: (1) The mechanism of melatonin suppression of estrogen and progesterone receptors in hamster uteri will be investigated. (2) Daily injections of melatonin will be administered to rats after tumor development to determine if tumor regression can occur.

In vitro: (1) The mechanism of the generation of the melatonin rhythm in vitro will be pursued. (2) Hamster pituitaries will be incubated with melatonin to determine if this hormone can suppress prolactin secretion.

In human subjects: (1) Melatonin profiles will be followed in subjects at each stage of puberty. (2) Obese patients will be followed during the course of weight loss to determine if any changes occur in the daily plasma melatonin profile. (3) More breast cancer subjects will be studied.

Publications:

1. Perlow, M.J., Reppert, S.M., Tamarkin, L., Wyatt, R. J., and Klein, D.C.: Photic regulation of the melatonin rhythm: Monkey and man are not the same. Brain Research 182: 211, 1980.

2. Tamarkin, L., Reppert, S.M., Klein, D. C., Pratt, B., and Goldman, B.D.: Studies on the daily pattern of pineal melatonin in the Syrian hamster. Endocrinology, in press.

3. Tamarkin, L., Reppert, S.M., Orloff, D. J., Klein, D. C., Yellon, S.M., and Goldman, B.D.: Ontogeny of the pineal malatonin rhythm in the Syrian (Mesocricetus auratus) and Siberian (Phodopus sungorus) hamsters, and in the rat. Endocrinology, in press.

4. Roselle, D., Tamarkin, L., Chabner, B., Lippman, M., and Cohen, M.: Melatonin suppresses and pinealectomy enhances dimethylbenzanthracene breast carcinogenesis in the rat. American Society for Clinical Investigation, 1980 Annual Meeting.

5. Tamarkin, L., Ehrenkranz, J.R.L., and Sidbury, J.B.: Circadian patterns of melatonin in normal children, exogenous obesity and Prader-Willi syndrome patients. Pediatric Research. 14, 484 (1980).

6. Ehrenkranz, J.R.L., Tamarkin, L., Bybee, D.E., Johnsonbaugh, R.E.: Daily pattern of plasma melatonin in precocious puberty, Endocrine Society, 1980 Annual Meeting.

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ANNUAL REPORT
of the
Endocrinology and Reproduction Research Branch
National Institute of Child Health and Human Development
October 1, 1979 through September 30, 1980

The Endocrinology and Reproduction Research Branch conducts research into the basic and clinical aspects of endocrine regulation and the control of reproductive function. In addition, the Branch provides training for Clinical and Research Associates, and Visiting Fellows and Associates, in laboratory and clinical investigation. This includes participation in the combined endocrinology training program with NIAMDD for Clinical Associates, and with the Developmental Endocrinology and Pregnancy Research Branches in the Fellowship program in reproductive endocrinology between NIH and the Armed Forces Institute.

During the current year, research has continued on the basic programs of the Branch, including the analysis and characterization of protein hormones and their cellular receptors, the structure-function relationships of peptide hormones, the regulation of pituitary hormone biosynthesis and secretion, and the study of mechanisms of hormone action. Other studies included the continuing analysis of pituitary-gonadal relationships, and of the regulation of ovarian function during the reproductive cycle and pregnancy, with particular emphasis on the participation of hormone receptors in the control of gonadal function. Further work was performed upon the analysis of pituitary-adrenal regulation, and in several areas of clinical investigation. This report describes the recent progress in research on the characteristics and secretion of peptide hormones, their interactions with cellular receptors and mechanisms of target cell action, the control of steroid hormone biosynthesis and secretion, and the role of hormones in cellular regulation and clinical disorders.

The Section on Biophysical Endocrinology conducts research on the mathematical and statistical analysis of endocrine regulatory processes and the properties of protein hormones and other ligands, studied by electrophoretic techniques, mathematical modeling, computer simulation, and biophysical methods during studies on ligand-receptor interactions, regulation of the ovarian cycle, and cardiovascular changes in thyroid disease.

A correlated series of experimental and theoretical studies on hormone-receptor interactions was performed in several model systems, including receptors for adrenal steroids, opiates, benzodiazepine, GnRH, and insulin. Characterization of these ligand-receptor pairs has been facilitated by the development of appropriate mathematical models, and computer programs for curve fitting and data analysis. New methods were developed to characterize ligand binding systems in which multiple labeled ligands react with multiple classes of sites, by analysis of binding-inhibition curves for a variety of unlabeled analogs, and to analyze binding isotherms in terms of the limiting slopes or intercepts of the corresponding Scatchard plots or dose-response curves. Other methods were developed to characterize binding systems in terms of a continuous distribution of affinities, or in terms of a discrete affinity distribution by setting the binding affinity to a pre-selected value based on prior experimental data. These approaches should greatly facilitate the characterization of steroid hormone receptors and their screening in breast cancer and other hormonally responsive tumors. Simplified

computer programs have also been developed for obtaining initial estimates of binding parameters with a small programmable calculator, and for statistical estimation of binding affinity, capacity and cooperativity factors in systems exhibiting cooperative binding properties.

In a related area, the mathematical and statistical theories of radioimmunoassay have continued to be subjected to progressive development, refinement, and testing. Particular attention has been given to recently introduced assay methods such as the immunoradiometric and enzyme-mediated techniques, and the chemical and mathematical equations describing these systems have been defined and used for computer simulation and data analysis. The mathematical theory for optimization of RIA has been extensively tested in practice and shown to be valid and useful guides to experimental techniques. New statistical methods for assay of radioassay data, and new computer programs for quality control of RIA, were developed to facilitate data processing and comparisons both within laboratories and in world-wide collaborative studies.

The Electrophoresis Unit conducts research on the development and application of electrophoretic techniques for the fractionation and isolation of peptides and proteins of biological interest. Methods such as analytical polyacrylamide gel electrophoresis (PAGE), electrofocusing, and isotachophoresis are employed for the characterization and isolation of hormonal and other regulatory proteins. Current research has focused on the standardization of laboratory procedures for the several forms of PAGE electrophoresis and focusing, and for re-stacking of protein zones to facilitate the purification of hGH isohormones and hCG. Also, polymers such as Sephadex and agarose were employed in electrofocusing and protein extraction to improve the efficacy of these fractionation and isolation steps. Electrofocusing methods on Sephadex and polyacrylamide gels were employed for the purification of serum macrophage stimulating factor, immunoglobulins, and hGH isohormones. The analysis of IgG patterns by PAGE and electrofocusing provides a magnified view of the IgG species that should facilitate the identification of variations and/or abnormalities of IgG in specific disease states. The application of electrofocusing to protein separation was improved by the use of an optimally flattened pH gradient, applied to the separation of hGH isohormones in milligram amounts. These species were also resolved by isotachophoresis, with improved load capacity and yield by comparison with electrofocusing. The application of electrophoretic methods to separation of cells and subcellular elements was also explored, with promising results from the use of non-denaturing detergents for the fractionation of active proteins. The basic aspects of gel electrophoresis were further explored by application of continuous optical scanning during polyacrylamide gel electrophoresis. This approach permits estimation of the apparent diffusion constant, and has been extended to studies on the properties of detergent micelles, the analysis of ragweed pollen antigens and the comparison of electrophoresis in polyacrylamide and agarose gels using ACTH and ragweed pollen antigens as model compounds.

The Section on Molecular Structure conducts research on the analysis, synthesis, and structure-function relationships of biologically active peptides. This includes the identification and synthesis of unusual structure and sequences in amino acids and peptides, and the development of new techniques for peptide sequencing and synthesis. Correlations between peptide structure and function are analyzed in hypothalamic releasing hormones, chemotactic and ion-channel forming peptides, and peptides which inhibit fetal and tumor growth.

Analysis of the peptides Cinnamycin and Duramycin revealed the presence of hydroxyaspartic acid, identified as dehydroaspartic acid in hydrolysates of the two peptides. Other unusual amino acids found in these peptides include: lanthionine, β-methyl-lanthionine, and lysinoalanine. Another analytical study was performed to develop conditions for the separation of hydroxylysine and its glycosides in the amino acid analyzer to facilitate diagnosis and clinical studies on osteogenesis imperfecta.

Synthesis of biologically active peptides included gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone releasing hormone (LHRH) on LH releasing factor (LRF), and its agonist analogs. The experience gained in this program will be applied to synthesis of more highly active GnRH analogs, and antagonist derivatives of the releasing hormone. These will be evaluated by in vitro and in vivo assays for receptor binding activity and gonadotropin release from cultured pituitary cells. Other peptides synthesized included thyrotropin releasing hormone (TRH) for clinical studies, analogs of the linear gramicidins, heterodetic multicyclic peptides such as nisin, and chemotactic peptides with agonist and antagonist activity.

The Protein Chemistry Unit conducts research on the chemical and biological properties of gonadotropins in tissues and body fluids, with emphasis on the isolation, structure-function relationships, and assay of gonadotropins. Current studies have verified the presence of an hCG-like hormone in the human pituitary gland, with physicochemical, immunological, and biological features typical of hCG. The pituitary gland contains the highest amount of 'hCG' among non-pregnant tissues, suggesting that the hCG-like hormone could be a precursor of LH in the human pituitary gland.

Analysis of the antigenic sites of the hCGβ subunit by assay with antisera to peptide analogs of the subunit showed the presence of three major antigenic determinants. These were the terminal dipeptide, Pro-Gln, the residues 133-136 (Arg-Leu-Pro-Gly), and residues 111-115 (Asp-Asp-Pro-Arg-Phe). The latter sequence as it exists in hCG or the hCGβ subunit molecule is not accessible for binding to these hCG-specific antisera. The relatively low titer and binding affinity of antisera to the carboxypeptide-terminal sequence (in comparison with anti-hCG and anti-hCGβ) are attributable to the limitation of antigenicity in the carboxyterminal sequence.

The isolation and purification of gonadotropins for assay or characterization has been facilitated by the use of a simplified elution method for dissociation of glycoproteins from lectin affinity systems such as agarose-Concanavalin A. This depends upon the use of 1 M NH_4OH instead of the usual sugar solutions employed to elute bound gonadotropins from the affinity gels. The method is simple and rapid, and permits recovery of up to 97% of the active hormone and subsequent lyophilization due to the volatile nature of the eluant. This procedure has proven useful for the extraction of gonadotropins from pregnancy urine of women and primates, and for concentration of the hormones from urine or plasma prior to measurement by radioimmunoassay.

The Section on Hormonal Regulation performs research on the control of endocrine target cells by peptide hormones, in particular the regulation and activation mechanisms of membrane receptors for gonadotropins, lactogens, angiotensin, and gonadotropin-releasing hormone (GnRH). Further studies were performed on the characterization of receptors for gonadotropins, prolactin,

and TSH, the latter in thyroid membranes. Analysis of highly purified bovine LH and TSH in receptor assays showed that TSH does not bind to testis LH receptors, whereas bovine LH possesses a small amount of intrinsic TSH-binding activity in the thyroid gland. Similarly, studies on the TSH-competing activity excreted by patients with metastatic choriocarcinoma provided further evidence that hCG acts as the abnormal thyroid stimulator in such cases by interacting with the TSH receptor sites. The intrinsic TSH-like activities of both LH and hCG can be attributed to structural similarities that permit binding of the gonadotropin molecules to the thyroid TSH receptors.

Regulation of testicular LH receptors was demonstrated in the primate testis, together with down-regulation by administered hCG, and the appearance of a rate-limiting steroidogenic lesion. In the rat testis, regulation of LH receptors was accompanied by marked changes in prolactin receptors, and a similar relationship was demonstrated in the ovary and adrenal gland during trophic hormone stimulation. These findings suggest that turnover of prolactin receptors is an important concomitant of activation of the target-cells by trophic hormones. Testicular gonadotropin receptors were also shown to be directly regulated by the hypothalamic peptide, GnRH. This finding, and similar results in the ovary, explain some of the paradoxical inhibitory effects of GnRH and related peptides upon gonadal function. In the ovary, GnRH and its agonist analogs inhibited the ability of hCG and epinephrine to stimulate progesterone production and cyclic AMP formation. The specific high-affinity receptors for GnRH in the ovary appear to exert their inhibitory effect on luteal cell function by impairment of cyclic AMP formation, with consequent reduction of progesterone production and secretion. GnRH agonists also caused marked inhibition of granulosa cell maturation and follicular luteinization in vivo, again by a direct action on the ovary.

The regulation of pituitary function was studied in dispersed pituitary cells, in which GnRH receptor occupancy was coupled to release of gonadotropins. The actions of GnRH upon gonadotropin release were highly dependent upon calcium, but did not involve the production of cyclic AMP. Stimulation of cyclic GMP formation by GnRH in cultured pituitary cells was also calcium-dependent, but was not an essential component in the action of GnRH, since inhibition of cyclic GMP formation by mycophenolic acid did not inhibit the gonadotropin response to GnRH. Pituitary GnRH receptors were also employed for radioligand-receptor assay of GnRH agonist and antagonist analogs, showing a positive correlation between receptor binding affinity and relative biological activity of the several analogs tested. This assay provides a rapid method for analysis of structure-function relationships in GnRH peptides, and for evaluating the contribution of receptor binding affinity to the actions of new GnRH analogs. Pituitary GnRH receptors were also analyzed during development and compared with the pituitary and serum levels of gonadotropins and steroids. GnRH receptor concentration reached a maximum value at three to four weeks of age and gradually declined to a minimum. The close correlation between GnRH binding, pituitary LH content, and serum gonadotropins, indicated the physiological importance of the GnRH receptors during development.

The control of angiotensin II receptors and aldosterone production was studied in the rat adrenal gland. The ability of angiotensin II to increase its glomerulosa-cell receptors and the enzymes of the aldosterone biosynthetic pathway was consistent with the role of angiotensin II as a major regulator of adrenal function. A requirement for angiotensin II was demonstrated in the normal increases in adrenal angiotensin receptors and aldosterone secretion that

accompany sodium restriction, by inhibition of converting enzyme activity, and consequently of angiotensin II formation, during decreased sodium intake. During normal sodium intake, inhibition of converting enzyme activity also caused a decrease in adrenal angiotensin receptors and plasma aldosterone, indicating the primary role of angiotensin II as a trophic hormone of the zona glomerulosa. Stimulation of aldosterone production by angiotensin II did not depend on cyclic AMP production, whereas calcium uptake was found to be an important component in the steroidogenic responses to both angiotensin II and potassium. The regulation of angiotensin II receptors was also studied in uterine smooth muscle of rat and rabbit during the estrous cycle and pregnancy. Uterine angiotensin II receptors were increased by estrogen and decreased by progesterone, and showed a marked increase during the first half of pregnancy, when the sites were mainly localized in the implantation area. Analysis of smooth muscle receptor regulation was commenced in the mesenteric artery to characterize vascular responses, and will be extended to changes during altered sodium intake and experimental hypertension.

The Section on Molecular Endocrinology investigates the molecular basis of peptide hormone action, with particular emphasis on the characterization of gonadotropin receptors, activation of steroid biosynthesis in gonads and adrenal, and analysis of the biological activity of circulating gonadotropins. The characterization and purification of LH receptors was pursued in membrane fractions from the rat ovary, employing Triton X-100 and low ionic strength buffers. Affinity chromatography on Sepharose-Concanavalin A gave four peaks of receptor activity which were further characterized by polyacrylamide gel electrophoresis. The affinity of the purified receptors was 0.3×10^{10} M^{-1} and the binding capacity was 4,200 pmol/mg, equivalent to about 63% purity. These studies suggested that the receptor possesses a subunit structure, with variable degrees of association within the cell membrane. Specific receptors for prolactin and other lactogens were also characterized in the rat ovary, and shown to be extractable in high yield with non-ionic detergents, with a significant increase in binding capacity above that of the particulate preparation. Further studies on the purification and properties of gonadal adenylate cyclase revealed stimulation of the testicular enzyme by LH and hCG in the presence of guanyl nucleotides, associated with binding of tritiated Gpp(NH)p. The hormone-induced increase in nucleotide binding was due to increased availability of nucleotide binding sites, and the ED_{50} for adenylate cyclase activation by LH was similar to that for gonadotropin-induced binding of the GTP analog. Such gonadotropin-stimulated binding of Gpp(NH)p in Leydig cell membrane may represent interaction with the guanyl nucleotide regulatory site during hormonal activation of adenylate cyclase.

In the intact Leydig cell, hCG and cyclic AMP were found to induce a cascade of protein phosphorylation that was correlated with hormone-stimulated increases in cyclic AMP and the subsequent elevation of testosterone production. In the adrenal fasciculata cell, two species of cyclic AMP-dependent protein kinase holoenzymes were characterized with molecular weights of 160,000 and 106,000. During ACTH action, rapid cyclic AMP-dependent phosphorylation by protein kinase was observed in thirteen cytosolic proteins, indicating the involvement of multiple phosphorylation events in the stimulation of adrenal steroidogenesis. The steroid biosynthetic lesion in gonadotropin-desensitized Leydig cells was further studied, and evidence for a role of estradiol in the enzymatic block following gonadotropin stimulation *in vitro* was suggested by rapid increases in testicular estradiol and concomitant nuclear translocation of estradiol receptors. These findings, and the ability of Tamoxifen, an estrogen antagonist, to block the steroidogenic lesion, indicate that the defect in androgen synthesis

at the desmolase and 17α-hydroxylase steps results from stimulation of endogenous estrogen production by gonadotropin.

The analysis of immunoactive and bioactive serum LH in the rat was performed by optimized assays of increased sensitivity. Bioactive serum LH in adult male rats was 10-15 times lower than in men, and 4-5 times lower than in the rhesus monkey. After castration, the B:I ratio of serum and pituitary LH decreased, suggesting a requirement for steroid in the maintenance of optimal biological activity. This was confirmed by replacement studies in which testosterone and dihydotestosterone were shown to prevent the decreased B:I ratio of serum LH. Estrogen was also found to affect the B:I ratio of serum LH, probably through decreased secretion rate as well as a possible direct effect at the pituitary level. In the female, LH bioactivity increased markedly at the time of the mid-cycle LH surge, indicating that the preovulatory LH peak is released from a pituitary pool of high biological activity. These studies will be extended to analyze the molecular basis of the steroid effect upon LH bioactivity, and the possible physiological implications of this change during the reproductive cycle.

The Metabolism Unit studies the regulation and hormonal control of glycogen metabolism in normal and diabetic tissues, and the mechanisms by which protein kinase and phosphoprotein phosphatase regulate the activity of glycogen synthase. Recent studies on the phosphorylation of glycogen synthase from rabbit skeletal muscle by cyclic AMP-independent synthase kinase have shown that phosphorylation at up to four sites occurs in a sequential order, in which one site has to be phosphorylated before the other three sites are phosphorylated. Analysis of the functional roles of these multiple phosphorylation sites revealed that one site primarily controls the binding of substrate, UDP-glucose, and three sites control the binding of the allosteric activator, glucose-6-phosphate, to the synthase. In contrast, cyclic AMP-dependent protein kinase incorporates up to 1.5 mol phosphate per glycogen synthase subunit, shown to occur at two sites by analysis of phosphorylated tryptic peptides. These sites affect the binding of UDP-glucose to the synthase, but have little effect on the binding of glucose-6-phosphate. One of the sites phosphorylated by cyclic AMP-dependent protein kinase, and three of the sites phosphorylated by cyclic AMP-independent synthase kinase, were found to be dephosphorylated by human placental and calf intestinal alkaline phosphatase. These enzymes will be useful for analysis of the functional roles of the phosphorylation sites in glycogen synthase and other phosphoproteins. Glycogen synthase provides a valuable model for investigation of the regulation of phosphorylation and dephosphorylation reactions that are key processes in the control of gene expression, cellular metabolism, differentiation and growth. Also, identification of the functional roles of the several phosphorylation sites of the enzyme should clarify the functional changes in glycogen synthase associated with fetal development and diabetes.

The Adrenal Research Unit investigates the physiology and regulation of adrenal steroidogenesis, by characterization of cellular steroid-binding proteins, and soluble factors which mediate steroidogenic responses to ACTH, and analysis of cellular mechanisms of cholesterol utilization in steroid biosynthesis. Studies were continued on the development of improved radioimmunoassay and bioassay methods for circulating ACTH, and comparison of the steroidogenic activity of cultured adrenal cells from rat, monkey, and guinea pig revealed a striking difference in sensitivity to ACTH between these three species. The role of non-catalytic steroid-binding proteins in adrenal steroidogenesis was

further investigated, by studies on the six specific binding proteins previously identified for cholesterol, 20α-hydroxycholesterol, pregnenolone, pregnenolone sulfate, and 21-hydroxypregnenolone. The role of microtubules in adrenal steroid synthesis was further examined, and purification of microtubules from bovine brain and adrenal cortex was performed. Further analysis of the microtubular and microfilamentous systems of the adrenal cortex will be performed in a stable, homogeneous population of cultured adrenocortical cells. A soluble stimulatory factor detected in the adrenal cortex of hypophysectomized rats following ACTH treatment was found to cause increased pregnenolone formation in adrenal mitochondria. This factor has been partially characterized, and will be further purified to elucidate its nature and function.

Clinical studies included the continuing evaluation of adrenocortical hyperfunction and a randomized study for treatment of pituitary chromophobe adenomas by irradiation. Also, the value of blood samples collected from the inferior petrosal sinus for diagnosis and treatment of pituitary tumors was further investigated. Studies on the use of the QKD interval as a non-invasive indicator of thyroid hormone and β-adrenergic actions on cardiovascular function were continued. The method was applied to evaluation of thyroid function in children with cystinosis, who were found to be generally euthyroid. The technique was also used to evaluate the effect of fasting on the thyroid status of patients with morbid obesity, and was correlated with the results of thyroid hormone assays. The effect of temperature and other physical factors on localization, natural history and susceptibility to disease was evaluated, with particular reference to the local effects of various tropical and metabolic disorders. The possible relevance of such factors to the use of antipyretic therapy, and the susceptibility of the testis and adrenal to certain infectious diseases, will be further evaluated by studies relating local body temperature to the site of active lesions in a variety of disease states.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00021-04 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Bioassayable and Radioimmunoassayable ACTH

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	ERRB, NICHD
	Kevin Catt	Chief	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two independent methods of characterizing and quantitating ACTH from clinical samples are being developed, a classical radioimmunoassay with materials supplied by the NIAMDD anmd a bioassay, using as an endpoint the corticosterone production of isolated rat adrenal fasciculata cells. Both assays have been found to be sensitive enough for human use and to have specificity which is suitable. An extraction procedure is being developed and is the final step in completing both assays.

In addition gel chromatography and ion exchange chromatography are being used to characterize the ACTH. Also, the columns are being used to prepare biologically active ^{125}I-labelled ACTH for receptor studies.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00022-07 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Renin-Angiotensin System and Aldosterone Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This program is directed at the elucidation of the role of the renin-angiotensin system and other factors in the regulation of aldosterone secretion by the zona glomerulosa of the adrenal gland. Specific areas of research include the characterization and regulation of angiotensin II receptors, the actions of angiotensin II and other regulators of aldosterone secretion, and the production and metabolism of angiotensin II. Major emphasis has been placed upon the use of enzyme-dispersed adrenal cells for analysis of the relations between angiotensin II receptors and aldosterone production, and the interactions between angiotensin II and other factors such as ACTH, sodium and potassium levels. The effects of altered electrolyte balance on adrenal receptors and steroid responses are studied, in particular the effects of sodium intake on the concentration and affinity of angiotensin II receptors in adrenal and smooth muscle cells. These studies are designed to clarify the control of adrenal and vascular sensitivity to angiotensin II during sodium deficiency, the mechanisms by which angiotensin II regulates the concentration of its receptors in adrenal and smooth muscle, and the activation pathways that are initiated by occupancy of angiotensin II receptors.

Objectives:

This project aims to clarify the role of the renin-angiotensin system in the regulation of aldosterone secretion in physiological and pathological conditions. This includes characterization of the properties and regulation of angiotensin II receptors, and their role in the activation of adrenal steroidogenesis; elucidation of the mechanisms of action of angiotensin II and other regulators of aldosterone secretion in the zona glomerulosa; comparison of the angiotensin II receptors of adrenal and smooth muscle, and their regulation by electrolyte changes and angiotensin II; and evaluation of the role of the renin-angiotensin system and aldosterone in certain forms of clinical hypertension.

Methods Employed:

Radioimmunoassays of renin activity, angiotensin II, aldosterone, corticosterone, cortisol, and cyclic nucleotides. Binding studies with ^{125}I- and ^{3}H-angiotensin II in adrenal cells and membranes, and uterine or vascular membranes, enzymatic dispersion of adrenal glomerulosa and fasciculata cells. In vivo and in vitro stimulation of aldosterone responses by rat and dog adrenal cells. Measurement of agonist and antagonist activities of angiotensin II fragments and analogues upon receptor binding and aldosterone production in vitro.

Major Findings:

1. Regulation of angiotensin II receptors and steroidogenesis in adrenal zona glomerulosa cells.

   (a) Regulatory actions of angiotensin II on adrenal cells.

The trophic effects of angiotensin II (AII) on adrenal glomerulosa function were studied by prolonged infusion of the Ile^{5}-octapeptide in intact and 14-day hypophysectomized rats. The administration of a non natriuretic dose of AII (50 ng/ min) for 6 days elevated blood levels of AII 3-fold and increased the plasma aldosterone concentration from 9.6 ± 1.2 to 29.7 ± 5.7 ng/dl in intact rats and from 7.5 ± 1.5 to 31.6 ± 7.4 ng/dl in hypophysectomized rats. AII receptors measured in adrenal glomerulosa fractions from hypophysectomized rats were increased 2-fold, from 1408 ± 138 to 2270 ± 324 fmol/mg. Significant increases in enzymes of the aldosterone biosynthetic pathway, more marked in hypophysectomized animals, were observed after AII infusion. Mitochondrial cholesterol content and pregnenolone formation were increased by 30% in intact rats and by 100% and 240%, respectively, in hypophysectomized rats. The conversion of corticosterone to aldosterone was also increased, by 100% in intact rats and by 180% in hypophysectomized rats. Microsomal cytochrome P-450 and 21-hydroxylase activities did not change after AII infusion in intact rats but were increased by about 100% in hypophysectomized rats. There was also an increase of 35% in 11-hydroxylase activity in hypophysectomized rats after AII infusion. These data show that the trophic actions of AII on the adrenal

glomerulosa zone are manifested by increases in both AII receptors and enzymes of the steroid biosynthetic pathway, and indicate the importance of AII as a major regulator of adrenal function during altered sodium intake.

(b) Regulation and properties of adrenael angiotensin II receptors during sodium restriction.

The properties of angiotensin II receptors and their role in mediating aldosterone secretion were analyzed in adrenal glomerulosa cells from normal and sodium-restricted rats. Adrenal receptors for angiotensin II were modulated by changes in sodium intake, with consistent increases after 36 hours of sodium restriction. Conversely, glomerulosa cell receptors for angiotensin II were decreased after 36 hours of high sodium diet. At each extreme of sodium intake, the change in angiotensin receptors was accompanied by a corresponding shift in adrenal sensitivity to angiotensin II, with increased or decreased aldosterone production by glomerulosa cells in vitro during stimulation by the octapeptide. The increases in adrenal receptors and aldosterone responses during sodium restriction were shown to be attributable to the trophic effect of angiotensin II on the zona glomerulosa cells by two approaches. First, infusion of angiotensin II into normal rats was found to increase adrenal angiotensin receptors and the magnitude of glomerulosa cell responses to angiotensin stimulation. Second, blockade of angiotensin II formation by infusion of the converting enzyme inhibitor, SQ 14,225, prevented the increases in adrenal receptors and aldosterone secretion that normally accompany sodium restriction. In animals on normal sodium intake, inhibition of converting enzyme activity also caused a fall in blood angiotensin II, adrenal angiotensin receptors, and plasma aldosterone. These findings have demonstrated that angiotensin II has a primary role in the control of aldosterone secretion, both as an acute stimulus and as a long-term regulator of glomerulosa cell function. It is concluded that the renin-angiotensin system acts as the major determinant of aldosterone secretion during physiological responses to altered sodium intake.

2. Role of calcium and cydic AMP in adlosterone production by zona glomerulosa cells.

(a) Dependence of Aldosterone responses on calcium uptake.

The role of calcium uptake in aldosterone production by isolated adrenal glomerulosa cells was analyzed during stimulation of steroidogenic responses by angiotensin II, potassium and ACTH. The calcium uptake inhibitors, lanthanum and verapamil, both reduced the stimulation of aldosterone production by each regulator in a dose-dependent manner. Each of the calcium antagonists blocked the stimulation of aldosterone and cyclic AMP production by ACTH, the degree of inhibition being dependent on the concentration of ACTH employed. Increasing concentrations of verapamil increased the ACTH concentration required for half-maximal stimulation, as well as reducing the maximum production of aldosterone. Inhibition of calcium uptake by lanthanum and verapamil also decreased steroid production by glomerulosa cells in response to angiotensin II or potassium, but did not alter the concentration of angiotensin II required for half-maximal stimulation of aldosterone production. Pregnenolone synthesis

stimulated by angiotensin II was also inhibited by verapamil, indicating that calcium is required for the action of angiotensin II at an early step in the steroidogenic pathway. The calcium antagonists had no effect on binding of angiotensin II to glomerulosa cells, demonstrating that the calcium requirement occurs at a post-receptor locus. These results provide further evidence that angiotensin II and potassium increase aldosterone production in adrenal glomerulosa cells through a calcium-dependent mechanism, and indicate that calcium uptake is an essential requirement for the stimulation of aldosterone production by these two effectors.

(b) Role of cyclic AMP in aldosterone responses.

The role of cyclic AMP in the regulation of aldosterone production by adrenocorticotropic hormone (ACTH), angiotensin II (AII), potassium, and serotonin was examined in collagenase-dispersed adrenal glomerulosa cells. The ability of 8-bromo cyclic AMP and choleragen to stimulate maximum aldosterone production indicated that cyclic AMP could act as second messenger for certain of the aldosterone-stimulating factors. The actions of ACTH and choleragen on aldosterone and cyclic AMP production were correlated in dog and rat cells, and a similar relation was seen during stimulation of rat cells by serotonin. In contrast, A II and potassium did not cause changes in cyclic AMP formation while stimulating aldosterone production. Intracellular and receptor-bound cyclic AMP were increased 3-fold by 10^{-7} M ACTH but not by AII. Addition of a phosphodiesterase inhibitor increased the magnitude of the cyclic AMP response to ACTH but did not change the lack of stimulation by AII or potassium. In dog cells, the effects of AII and potassium on aldosterone production were partially additive to those of ACTH, choleragen, and 8-bromo cyclic AMP. In contrast, no additivity was observed between AII and potassium, or between combinations of the cyclic AMP-dependent stimuli. These results indicate that the actions of ACTH on aldosterone secretion are mediated by cyclic AMP formation,whereas AII and potassium stimulate aldosterone production through an independent mechanism. The lack of additivity between steroid responses to AII and potassium suggests that these factors could share a common mode of action on steroidogenesis in zona glomerulosa cells.

3. Regulation of uterine angiotensin II receptors.

(a) Effects of estergen and progesterone.

The characteristics of uterine receptors for angiotensin II and their regulation during the estrous cycle and after gonadal steroid treatment were investigated in the rat and rabbit. Binding of [^{125}I]iodoangiotensin II to rat uterine particles was temperature-dependent and saturable, and Scatchard analysis showed a single population of binding sites with Kα of about $10^{9}M^{-1}$. During the ovarian cycle, the concentration of angiotensin II receptors was about 4 times higher at proestrus than at diestrus II. Ovariectomy was followed by a progressive decrease in uterine angiotensin II receptors, reaching 50% of the control values 8 days after castration. Conversely, injection of 17β-estradiol caused a dose-dependent increase in angiotensin II receptors after 24 h and,

short term infusion of 17β-estradiol increased angiotensin II receptors to a maximum of 4- to 6-fold above control values at 36 h. Progesterone infusion for 7 days caused uterine angiotensin II receptors to decrease by 93%. No effects of steroids upon the affinity of the receptors for angiotensin II were detected. These studies revealed marked changes inthe concentration of angiotensin II receptors during the estrous cycle and after steroid treatment, similar to the control of receptors for other oxytocic hormones. These findings may explain the increased contractile sensitivity of estrogen-primed uteri to angiotensin II, and suggest a role for angiotensin II in the physiological control of myometrial activity.

(b) Angiotensin II receptors during pregnancy.

The concentration of uterine receptors for angiotensin II was investigated during pregnancy in the rat by measuring binding of [^{125}I]angiotensin II to uterine particles at each day of pregnancy. During the first half of pregnancy, the concentration of receptors rose by 3-fold from day 2 to day 9 and returned by day 12 to values similar to those of nonpregnant control rats. During the second half of pregnancy, uterine angiotensin II receptors remained below control values until 1 day after delivery. The major changes in angiotensin II receptor concentration took place in the implantation area, with little or no alterations in the adjacent myometrium.

When unilateral salpingectomy was performed on day 2 of pregnancy, the concentration of angiotensin II receptors was still increased on day 9 in the contralateral pregnant horns but did not change in the sterile horns. However, this increase was restored by induction of a deciduoma in the sterile horns. After bilateral salpingectomy, pseudopregnant rats failed to show the increase in concentration in uterine angiotensin II receptors between days 5-11, while such rats bearing a deciduoma behaved similarly to normal pregnant rats.

The marked changes in the concentration of uterine angiotensin II receptors during early pregnancy in the rat are mainly localized in the implantation area and appear to be related to the decidualization process. The low angiotensin II receptor concentration in the uterus at the end of pregnancy exclude a major role for angiotensin II in the process of parturition in the rat.

Significance:

These studies are providing information about the control of aldosterone secretion, and the role of the renin-angiotensin system and angiotensin receptors in the regulation of adrenal and smooth muscle function.

Proposed Course:

The role of calcium and phospholipid metabolites in angiotensin action, and the steroid biosynthetic changes induced by stimuli of aldosterone secretion, will be analyzed in more detail. The relations between changing sodium balance and angiotensin II receptors in adrenal and vascular muscle will be further

studied during acute sodium restriction. The contribution of both systemic and locally formed angiotensin II to circulating angiotensin levels and adrenal regulation will be examined, and further characterization of angiotensin II receptors will be performed by affinity-labeling and solubilization of adrenal and uterine receptors.

Publications

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Vinci, J. M., Horwitz, D., Zusman, R. M., Pisano, J. J., Catt, K. J. AND Keiser, H. R.: The effects of converting enzyme inhibition with SQ20,881 on plasma and urinary kinins, prostaglandin E, and angiotensin II in hypertensive man. Hypertensoin I. 416-426, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00035-08 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Structure and Function of Biologically Active Molecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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5	1	4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Peptides with unique structures show distinct biological properties. These studies are directed at the analysis and synthesis of molecular assemblies with rare amino acids, for instance (a) the synthesis of precursors of ring A of nisin, a peptide with α,β-unsaturated and thioether amino acids; (b) the action of peptide sturctures based on nisin and/or the linear gramicidins on neonatal and nioplastic tissue; (c) the synthesis of analogs of the gramicidins A, B, and C, and the correlation of their sturctures with ion-transport across lipid bilayers; (d) the synthesis of peptides with agonistic and antagonistic effects on fertility regulating hormones inclusive of the large-scale preparation of the luteinizing hormone releasing factor (LRF) for clinical purposes; (e) the exploration of dehydroalanine resins for the synthesis of peptide amides; (f) the synthesis of analogs of opioid peptides with agonist and/or antagonist properties; (g) the synthesis of analogs of the pentapeptide L-Phe-D-Leu-L-Phe-D-Leu-L-Phe with chemotactic and anti-chemotactic characteristics; (h) the structural investigation of the heterodetic pentacyclic peptides CINNAMYCIN and DURAMYCIN; (i) the development of new techniques for peptide synthesis, amino acid analysis and nonenzymatic cleavage of the peptide bond.

Project Description Z01 HD 00035-08 RR

Objectives:

Structural elucidation, chemical modification, and partial and/or total synthesis of biologically important peptides which: (a) incorporate unique structural features brought about by the presence of α,β-unsaturated and thioether amino acids; (b) represent portions of the sequence of hormones of the reproductive endocrine system and are useful for immunological investigations and applications; (c) display hormone releasing or hormone release inhibiting properties; (d) affect neonatal and neoplastic tissue; (e) show ionophoric characteristics and mediate the transport of ions across lipid bilayers and biological membranes; (f) are chemotactic or antichemotactic for neutrophils; (g) are structural variants of the naturally found amino acid assemblies with opiate like activities. Chemical, physical, and biological studies with membrane active peptides. Chemical modification of amino acids and development of nonproteolytic methods for the selective cleavage of the peptide bond. Isolation and characterization of subcellular constituents from developing tissue. Development of new approaches to the synthesis of peptides of physiological interest. Design, development, and implementation of new techniques and instrumentation in amino acid analysis and peptide synthesis. Development of amino acid analytical approaches for the detection of uncommon constituents in physiological fluids with the aim of providing a tool for the early diagnosis of pathological states.

Methods Employed:

Highly selective and thermostable proteases (e.g. thermolysin) in combination with newly developed nonproteolytic techniques for the structural elucidation of peptides with infrequently seen amino acids. Dehydroalanine linkage of amino acids to carriers in the solid phase synthesis of peptides and peptide amides. Alkylamidated residues of dehydroalanine to incorporate ω-amide functions in endo-positions of the peptide chain. Novel-type protecting groups meeting the requirement of highly selective removal in peptide synthesis. Techniques for the synthesis of amino acids with multiple centers of chirality under the conditions of stereochemical control. Advanced techniques for the separation of closely related molecular species and their analysis, e.g. specifically developed sample application and data acquisition in amino acid analysis; counter current distribution, conventional and high pressure liquid chromatography; electrophoresis; circular dichroism; polarimetry; mass spectrometry; nuclear magnetic resonance; X-ray analysis.

Major Findings:

Peptide Synthesis and Biology: (a) Luteinizing Hormone Releasing Factor (LRF) and analogs of the same synthesized by various techniques of peptide bond formation. The highly purified products meet all applicable chemical and physical criteria. Biologically, they are fully active and ready for clinical studies aimed at the induction of ovulation. The large scale synthesis of the decapeptides called foar the development and application of highly refined techniques of peptide synthesis to incorporate side chain functions, such as the guanido group or arginine and terminal amides. Activation and methods of

protection had to be modified for the incorporation of sensitive amino acid residues, such as tryptophan, tyrosine, and serine. Considerable experience has been gained with the improvements made in the approaches to the synthesis of these delicate peptide structures. It will bear its fruit now that amino acids are being assembled to give molecular species that are hyperactive or display antagonistic properties. (b) Analogs of the Linear Gramicidins: i. HCO $(L\text{-}Ala\text{-}Gly)_7$-L-Ala-$NHCH_2$ CH_2OH, a distinct departure from the amino acid compositions of the naturally occurring analogues, is a novel pentadecapeptide in which all amino acids with aromatic side chains have been replaced. the strictly observed pattern of alternating configurations of L- and D-amino acids has been broken for the first time and it has been demonstrated that it is possible to replace all D-amino acids by GLYCINE without adverse effect on the conformation of the β-helix, the prerequisite for channel formation and ion transport across lipid bilayers. The nonchiral amino acid glycine adequately and functionally substitutes for the D-amino acid residues of the naturally occurring analogs of the linear gramicidins. Present data are in support of the formation of a β-helical structure by HCO-$(L\text{-}Ala\text{-}Gly)_7$-L-Ala-$NHCO_2CH_2OH$ as indicated by the detection of single channel characteristics for the molecule once embedded in the lipid bilayer. ii. HCO-$(-L\text{-}Val\text{-}D\text{-}Val)_7$-L-Val-$NHCH_2CH_2OH$ and shorter chain segments of the molecule synthesized for studies on lipid bilayers and in experiments with neonatal and neoplastic tissue to probe for fetal resorption inducing and tumor growth inhibiting activity. Rational: the naturally occurring linear gramicidins display both activities indicated, however, not without side effects presumably due to the presence of the aromatic side chains. Such groups are not present in the newly designed molecule. Other analogs of the linear gramicidins prepared and investigated: formyl-$(L\text{-}tryptophyl\text{-}glycyl)$-$(L\text{-}tryptophyl\text{-}D\text{-}leucyl)_6$-L-tryptophylethanolamine, formyl-$(L\text{-}tryptophyl\text{-}D\text{-}leucyl)_7$-tryptophyl ethanolamide, and fragments, e.g. $(L\text{-}Phe\text{-}D\text{-}Leu)_2$-L-Phe, for the synthesis of formyl-$(L\text{-}phenylalanyl\text{-}D\text{-}leucyl)_7$-L-phenylalanyl-ethanolamine. iii. Synthesis of Thyrotropin Releasing Hormone (TRH) - pyro-Gly-His-Pro-amide - for clinical studies. iv. Synthesis of Heterodetic Multicycle Peptides: 1. Nisin. Approaches to the synthesis of the biologically active (induced fetal resorption; inhibition of the growth of tumor cells) H_2N-terminal fragment of nisin call for the individual structures. First requirements for the synthesis of the various rings were the successful access to the unusual structural feature seen in ring A of nisin. The α,β-unsaturated amino acid DEHYDROALANINE is incorporated into suitable peptide structures via β-elimination of the appropriate precursors properly incorporated in the correct position in the form of an O- or S-substituted derivative of serine and/or cysteine, respectively. Great difficulty is associated with the preservation of the chirality of meso-lanthionine to be placed into Ring A. The most promising route established forms a sulfonium salt from S-alkyl-cysteine and β-halo-alanine, that is to be decomposed subsequently to give the thioether amino acid (in meso-form) without sacrifice in chirality. v. Synthesis of radio-labelled formyl- and tert.-butyloxycarbonyl-$(L\text{-}phenylanalyl\text{-}D\text{-}leucyl)_2$-L-Phenylalanine (vide supra, the segments for the synthesis of analogs of the linear gramicidins) for studies in chemotaxis. The formyl derivative is an agonist, the tert.-butyl derivative an antagonist of chemotaxis (neutrophils). To establish the mechanism of action of the agonist and antagonist, p-halophenylalanine has been incorporated in different positions of

the peptides for catalytic dehalogenation to provide tritiom-labelled derivatives. vi. Synthesis of enkephalin analogs for the exploration of agonist and antagonist properties and for physicochemical investigations.

Analysis: Peptides - Cinnamycin and Duramycin. Hydroxyaspartic Acid-Dehydroaspartic acid. Hydrolysates of cinnamycin and duramycin contained a constituent amino acid of unknown identity that elutes between the erythro-and threoform of hydroxyaspartic acid. The unknown component was likely to be derived from hydroxyaspartic acid and possibly its dehydration product, namely dehydroaspartic acid. Comparison with an authentic sample confirmed the identity of the unknown constituent in the hydrolysates of cinnamycin and duramycin as dehydroaspartic acid. The relationship between hydroxyaspartic acid and dehydroaspartic acid (reversible formation via hydration and dehydration) may well be of physiological significance. Amino acids found in cinnamycin and duramycin and linked in other ways via β-elimination and addition reactions are lanthionine, β-methyllantionine, and lysinoalanine.

Analysis: Amino Acids - Osteogenesis imperfecta - Hydroxylysine. The special conditions developed for the separation of hydroxylysine and glycosides of this amino acids on the ion exchange column of the amino acid analyzer make possible the determination and quantitation of this amino acid and its conjugates. This facilitates the diagnosis of osteogenesis imperfecta and will aid future clinical studies.

Significance to Biomedical Research and the Program of the Institute:

The sequence of amino acids and the synthesis of peptides with unique structural features are prerequisites for the understanding of the effects exerted by certain of these molecules on endocrine function. Amino acid assemblies capable of controling the release of hormones are of foremost concern. The execution of well-controlled procedures of peptide synthesis provides molecular species for clinical applications aimed at fertility regulation.

Nisin, the H_2H-terminal fragment of nisin, and the linear gramicidins act on neonatal and neoplastic tissue by inducing fetal resorption in rodents and inhibiting the growth of tumor cells. The modes of action responsible for these effects are not known at present. Working hypotheses call for: (a) the addition of essential nucleophilic groups in the physiological environment across the double bond of unsaturated amino acids, such as dehydroalanine (cf. nisin and its fragments); (b) the disturbance of vital ion equilibria existing on either side of the biomembrane (cf. the ionophoric and transmembrane-channel forming properties of the gramicidins A, B, and C, and their synthetic analogs). α,β-Unsaturated amino acids may be vital to many physiological processes. The structural features seen in these unique amino acids are related to the amide and keto-function - both very well established structural elements of physiological consequence - and to the peptide bond - a linking feature of no lesser biological significance.

The amide function is seen at the carboxyl terminus of all structurally established hormone releasing factors. It is also found in the same position

of numerous other biologically active peptides. The group of neurohypophyseal peptides is a case in point. The use of dehydroalanine as vehicle for the attachment of peptides to carriers in solid phase peptide synthesis and the direct conversion of this amino acid to the amide function in exo- and endo-positions of the peptide chain are a development that benefited from the chemistry developed in this laboratory subsequent to the discovery of dehydro-alanine and dehydrobutyrine in nisin and subtilin.

The linear gramicidins are distinguished from all other known peptide structures by the regularly repeating pattern of L- and D-configurations. This gives rise to a unique hydrogen bond stabilized helix capable of embedding in lipid bilayers and biomembranes and the mediation of ion transport through the transmembrane channels formed. The effects of the native linear gramicidins and their synthetic analogs on neonatal and neoplastic tissue (vide supra) are believed to be the consequence of the physical properties indicated and brought about by the structural features intrinsic to these molecules.

The peptides for which chemotactic and anti-chemotactic properties have been demonstrated (vide supra) are partial sequences of analogs of the linear gram-icidins. Assuming a mode of action different from that involving ion transport only, more subtle details of the relationship between structure and chemotaxis are to be investigated subsequent to the incorporation of radiolabel (dehalo-tritiation of phenylalanine).

The synthesis of partial sequences of hormones (and other physiologically important amino acids assemblies) that are responsible for antigenic acitvity provides important tools for the purpose of early diagnosis (radioimmunoassay) under normal and/or pathological circumstances.

Proposed Course of Project:

Improvements in the gram-scale synthesis of hormone releasing and hormone release inhibiting factors for fundamental and clinical investigations. Synthesis of H_2N-terminal protected peptide amides with properties antagonistic to the release of luteinizing and/or follicle stimulating hormone. Synthesis of analogs of the linear gramicidins with and without radiolable for autoradio-graphic investigations and studies to elucidate the modes of action of these peptides in the various physiological effect they cause (action on neonatal and neoplastic tissue; embedding in membranes; exploration as chemotactic and/or anti-chemotactic agents). Synthesis of partial sequences and individual ring systems of nisin; exchange of amino acids in and incorporation of radio-lable into these peptides.

Synthesis of analogs of: (a) opioid peptides (enkephalin and endorphin) with the prime objective of providing antagonists; (b) chemotactically and anti-chemotactically active peptides with alignments of the amino acids that are patterned on partial sequences of the linear gramiciding. Incorporation of radiolabel (tritium via dehalotritiation) for the purpose of exploring proposed mechanisms of chemotaxis.

Development and improvement of: (a) methods for the synthesis of peptides; (b) the protection of the functional groups of amino acids (the large scale synthesis of the luteinizing hormone releasing factor is one of cases that made evident the inadequacies of existing protecting groups; in this particular situation none of the protections for the guanido groups of arginine proved satisfactory; ornithine, suitably protected at the δ-amino group was employed as precursor; a guanidination reaction was executed subsequent to deprotection; several of the protecting groups under consideration are based on the chemistry of thioether and α,β-unsaturated amino acids; (c) Non-proteolytic techniques that are either applicable in general to the fragmentation of peptides and proteins or are conceived for the specific needs of establishing rare structural features, such as those seen in cinnamycin and duramycin. Correlation of identical structural components (e.g. α,β-unsaturated amino acids, lanthionines, lysinoalanine, and hydroxyaspartic acid) in peptides (nisin, subtilin, cinnamycin and duramycin) of strikingly different origin by the pursuit of phylogenetic questions.

Isolation, purification, and characterization of peptides of natural origin and chemical synthesis of peptides for the study of their inhibitory potential on intrauterine growth. Systematic screening of compounds of different chemical structure for activities on neonatal and neoplastic tissue. Spectroscopic investigation (nuclear magnetic resonance with different nuclei and circular dichroism) of the linear gramicidins and fragments of nisin in their interaction with lipid bilayers of known composition and adequately analyzed biomembranes. Conductance measurements and detailed investigations of the ion transport brought about by the gramicidins A, B, and C and synthetic analogs with amino acids exchanges. Isolation and characterization of subcellular constituents from developing tissue. Improvements in the design and construction of amino acid analyzers and peptide synthesizers.

Publications:

Bamberg, E., Alpes, H., Apell, H.J., Läuger, P., and Gross, E. (1979) Formation of Ion-Transporting Channels by Analogs of Gramicidin A. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function, Pierce Chemical Company, Rockford, IL, pp. 629-634.

Schiffmann, E., Venkatasubramanian, K., Corcoran, B., Aswanikumar, S., Day, A., Freer, R.J., Gallin, J.I., Clark, R.A., Hirata, F., and Gross, E. (1979) Chemotactic Peptides as Probes of Molecular Events in Leukocyte Chemotaxis. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function, Pierce Chemical Company, Rockford, IL. pp. 731-741.

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Izumiya, N., Kato, T., Aoyagi, H., Shimohigashi, Y., Yasutake, A., Lee, S., Noda, K., and Gross, E. (1979) Synthesis of cyclo-Tetrapeptides, AM-Toxins and Analogs of CYL-2. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function, Pierce Chemical Company, Rockford, IL, pp. 439-444.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 0040-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Experimental and Theoretical Studies of Hormone Receptor Interaction and Response Coupling

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	T. Garcia	Guest Worker	ERRB, NICHD
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4.5	0.25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A correlated series of experimental and theoretical studies regarding hormone receptor interactions has been undertaken. A number of model systems have been utilized, including the receptors for aldosterone, glucocorticoids, enkephalin, opiates, benzodiazepines, LHRH, and Insulin. By development of appropriate mathematical models, computer programs for curve fitting and data analysis, and of improved experimental designs, we have been able to characterize several of these ligand-receptor systems. Special attention has been given to the cases of 1) multiple ligands reacting simultaneously with multiple classes of sites, 2) the presence of multiple sub-sites on receptor areas; 3) the interaction of ligand-binding with an ionophore; 4) the interaction of receptors for different ligands on the same cell membrane.

Objectives:

This project is intended to develop both experimental and theoretical methods for analysis of hormone, neurotransmitter, and drug binding to their receptors. Methods are under study for characterization of the ligand-binding system (in terms of binding capacities, affinities, cooperativity factors, and any complex interactions), and the coupling of binding in response of the target cell (ionophore activation, second messengers and related phenomena).

Methods Employed:

Laboratory technique for binding of peptide hormones, steroid hormones, drugs, and neurotransmitters to their naturally accurring receptors, using appropriately radioactively labeled ligands. Also techniques of mathematical modeling, computer simulation, curve fitting, and Monte Carlo methods.

Major Findings:

The aldosterone and glucocorticoid binding to toad kidney epithelial cells has been studied, in collaboration with Drs. C. Watlington, University of Virginia, and J . Handler, NHLBI. It is of interest that the low affinity binding site appears to correlate with physiological activity.

The benzodiazepine receptor has been characterized. A number of experimental factors modify the behavior of this receptor. In particular chloride, iodide, bromide, and a series of other anions modulate the affinity of this receptor. Likewise GABA increases the affinity of this receptor, primarily due to a decrease in the dissociation rate. The effects of iodide and GABA are additive. Biccuculine, a GABA antogonist, can reverse the affect of both GABA and to some extent, iodide. Certain ionophore blocking agents (DIDS, SITS, DNDS) also modulate the behavior of the benzodiazepine receptor, as do selected purine and purine-analogs. This appears to be an ideal model system for study of interaction of muliple receptors on the cell surface, especially since it appears that both benzodiazepine and GABA are interacting with the same chloride anion channel.

The opiate receptor: We are utilizing mathematical modeling studies, and new experimental procedures to attempt to characterize the heterogeneity of the opiate receptor in brain, vas deferens, and other tissues. This has required new experimental designs, involving use of multiple labeled ligand, and constructing displacement curves for a variety of unlabeled ligands which show specificity for various sub-types of the receptor. To assist the studies, new labeled analogs of both enkephalin and opiate alkaloids have been designed.

A newly developed program, designated "SCAFIT", enables us to characterize ligand-binding systems, where data are available using multiple labeled ligands reacting with multiple classes of sites, with displacement curves for a variety of unlabeled analogs. This has been used to

characterize the LHRH receptor on bovine and rat pituitary membranes. These studies have shown that two newly developed analogs, D-Ser^{6}(t-Bu) des-gly^{10} NETH and D-Ala^{6}-des-gly^{10} NETH compete for the same sites as native LHRH. Further, the receptors show severe heterogeneity, consisting of two classes of sites of widely differing affinities.

The affinity consistent for each of the three ligand reacting with both classes of sites have been estimated by least-squares procedures. The model has been used to make several predictions which are now being evaluated experimentally. Three studies indicate that the iodinated analogs serve as appropriate probes of the LHRH receptor.

The new method has been developed to characterize binding isotherms in terms of the limiting slopes and intercepts of the Scatchard plots or of the corresponding dose response curves (in terms of B/T vs T). Another new method has been developed to characterize binding systems in terms of a continuous distribution of affinities. This method, requiring a relatively simple and short computer program, should be widely applicable to characterization of a wide variety of systems. A third new method for characterizing ligand-binding systems has been developed: This describes the binding systems in terms of a "discrete affinity distribution". This may be implemented on computer programs "DISAFF", "SCAFIT", "SCAT1.BAS" or "SCATFIT". In this approach, one sets the K values equal to pre-selected values, based on previous experimental data. This reduces the number of parameters which must be estimated, and thus vastly facilitates the curvefitting procedures. These last three mentioned approaches should be extremely useful for characterizing steroid hormone receptor systems, particularly in the context of screening for estrogen, progesterone, androgen and glucocorticoid receptors in breast carcinoma and other hormonally responsive tumors.

Dr. A. K. Thakur has examined the graphical properties of Scatchard plots, dose response curves (B/T versus Total), and B/F versus Free for several important models: The Adair or stepwise model, the Statistical-mehanical model, the DeMeyts model, and heterogeneity of receptor (2 or more classes of sites). He has developed methods to obtain initial estimates of binding parameters (affinity, binding capacity, cooperativity factors) using a small hand-held programmable calculator. These methods have already proven to be enormously successful and popular.

Two computer programs have been developed for characterization of ligand binding systems which can be described by the "DeMeyts" model. This permits statistical estimation of the affinity, binding copacity, cooperativity factor, and nonspecific binding. Methods have been developed for combining results from multiple curves within an experiment, from multiple patients, and from multiple experiments in order to obtain the most reliable estimates of parameters.

Significance to Biomedical Research and the Program of the Institute:

Much current research interest centers around the binding of hormones,

drugs and neurotransmitters to their naturally occurring receptors. The models which have been developed here should be generally applicable to endocrinology and to neurobiology. Further, these models are now sufficiently well advanced, so that they can be used to make predictions in specific systems, and to assist significantly in the design of experiments to discriminate among the possible mechanisms of action.

Proposed Course:

We expect that the models currently under study will continue to evolve in collaboration with several experimentalists at the National Institutes of Health and throughout the country. The models will be progressively refined and used as guides to experimentation. As new experimental results become available, the models will be developed further. In this process, a series of computer programs will continue to be developed for routine use in analysis of binding systems. We expect to have considerable increased application of these models to systems such as protein and polypeptide hormones, e.g., angiotensin, catecholamines, and steroid receptors.

By design of new ligands (analogs of enkephalin, morphine, etc.) we expect to be able to generate new experimental results which can be used to test hypothesis generated on the bases of existing data and models. We believe that some of these new probes will produce new structure-activity information unobtainable by any other method.

Publications:

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Murakami, T., Brandon, D., Rodbard, D., Loriaux, D.L., and Lipsett, M.B.: Glucocorticoid Receptor in Polymorphonuclear Leukocytes: A simple method for leukocyte glucocorticoid receptor characterization. Journal of Steroid Biochemistry, 10: 475M-y481, 1979.

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Thakur, A.K., Jaffe, M.L., and Rodbard, D.: Graphical analysis of ligand binding systems: evaluation by Monte Carlo studies. Anal. Biochem. in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00041-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cardiovascular Effects of Thyroid, Hormones, Catecholamines and Cardiotoxic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
Other	R. Osburn		NNMC
	E. C. Ridgeway	Professor	Massachusetts, General Hospital

COOPERATING UNITS (if any)
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National Naval Medical Center, Bethesda, Maryland
Massachusetts General Hospital, Boston, Massachusetts

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.25

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of thyroid hormones and beta adrenergic agonists on target tissues may be evaluated by noninvasive monitoring of cardiovascular function. New techniques have been developed and evaluated for this purpose. The QKD interval, i.e., the interval between the onset of ventricular depolarization and the arrival of the pulse wave at the brachial artery, provides a rapid, innocuous, precise, reproducible, noninvasive technique for evaluation of thyroid cardiovascular and/or catecholamine status. This has been used for diagnosis and monitoring of therapy for patients with thyrotoxicosis, hypothyroidism, pheochromocytoma, and in the titration of effects of beta adrenergic blockers such as propranolol. This method has now been applied to evaluate thyroid function in children with cystinosis, and appears to indicate that patients are generally euthyroid. This technique has been used to evaluate the effect of fasting on the thyroid status of patients with morbid obesity, and correlated with results of T_4, T_3, and reverse T_3 measurements.

Objectives:

Develop and evaluate methods for noninvasive monitoring of thyroid status, catecholamine stimulation or blockade, and myocardial contractility in patients with drug-induced or other cardiomyopathies.

Methods Employed:

A new method has been developed called "Sphygmo-Recording" or "Blood Pressure Intervalometer". The electrocardiogram, phonoarteriogram, and the pressure from the blood pressure cuff are fed into a specially designed electronic apparatus which automatically displays the calibrated contour of the arterial pressure wave on the phase of a storage oscilloscope. This is then photographed by Polaroid camera. This electronic instrumentation has also been utilized for improved recording of heart sounds, carotid sounds, thyroid bruits and a variety of other cardiovascular signals.

Major Findings:

The QK_d measurements were studied serially in patients with morbid obesity during several successive control and fasting periods. The results suggest that there is a systematic shortening of the QK_d interval during fasting (PL.05). This appears to negate the possibility that these patients become acutely hypothyroid. Patients with thyrotoxicosis show a brisk response (of a 15-30 msec prolongation of the QK_d interval) to oral propanolol. However, it does not appear that these patients are more sensitive than normal subjects.

There has been some question regarding the thyroid status of patients with cystinosis. Accordingly, the QKD measurements have been obtained in a series of approximately 10 patients with this disease. In order to evaluate these, normal ranges needed to be established in children of the same age and/or of the same height, weight, and hence, physical dimension between the heart and the location of the transducer. This has led to the collection of data to evaluate the normal QKD interval in children and to study the relationship between height and QKD interval.

Significance:

Despite the proclivity of chemical tests (particularly radioimmunoassays) for thyroid hormones, there is no generally available, reliable measure of thyroid effects on the target tissues for routine clinical use. The present study provides one such measure of thyroid effects on the end organ. In addition, it is useful in monitoring of therapy for pheochromocytoma, and for beta adrenergic stimuli and blockers. This method is useful in evaluating the cardiotoxicity of Adriamycin and related cancer chemotherapeutic agents.

Proposed Course:

The use of this technique for monitoring thyroid status is now well established, but it retains research interest in special cases. Accordingly, this measurement is to be used selectively in special cases, where a reliable measure of end-organ function is necessary, in addition to measurement of plasma levels of thyroxine and tri-iodothyronine.

Publications:

Bercu, B. B., Haupt, R., JohnsonBaugh, R., and Rodbard, D.: The Pulse wave arrival time (QKD interval) in normal children. J. Pediatrics, 95: 716-721, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00042-05 RR

PERIOD COVERED

October 1, 1979 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mathematical and Statistical Theory of Gel Electrophoresis and Filtration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Andreas Chrambach	Senior Investigator	ERRB, NICHD
	Betty Chen	Staff Fellow	ERRB, NICHD
	C. Strott	Senior Investigator	ERRB, NICHD
Other	B. Cole	Computer Programmer	DMB, DCRT
	J. Poduslo	Staff Fellow	NINCDS
	P. Lambin	Predoctoral Fellow	Paris, France

COOPERATING UNITS (if any)

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Massachusetts Institute of Technology, Cambridge, Massachusetts
University Pierre et Marie Curie, Paris, France

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Endocrinology and Reproduction Research Branch

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NICHD, NIH, Bethesda, Maryland 20205

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1.5	1.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mathematical and statistical principles of polyacrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, gel filtration, density gradient sedimentation, and related physical-chemical techniques have been developed. This is to permit optimization of conditions for fractionation of protein and polypeptide hormones, proteoglycans, hormone receptors and other macromolecules. Methods and computer programs have been developed to permit improved statistical estimation of molecular weights derived from these techniques. Further, general computer programs have been developed for analysis of the concentration profiles which arise in electrophoresis, chromatography, sedimentation and other physical-chemical techniques. These programs should be extremely useful for measurement of diffusion coefficients, bandwidth, and resolution.

PHS-6040

Objectives:

Improvement of the performance of physical-chemical techniques used for the analysis and purification of protein and polypeptide hormones.

Methods Employed:

Polyacrylamide gel electrophoresis, gel filtration, sucrose density gradient sediment, sucrose density electrophoresis of cells and proteins, isoelectric focusing. In particular, polyacrylamide gel electrophoresis and isoelectric focusing is performed with continuous scanning of the concentration profile with computation of relevant molecular parameters.

Major Findings:

A new computer program has been developed for the automatic analysis of concentration profiles which arise in electrophoresis, chromatography, and sedimentation. Usually such profiles consist of one or several Gaussian peaks, superimposed on a base line. Algorithms have been developed for automatic, computerized peak detection. This is followed by characterization of the peaks in terms of the mean location (centroid or center of gravity) area and standard deviation. A nonlinear least-squares curve fitting program is utilized to describe the curves as the summation of several overlapping Gaussian components. This program, written in the BASIC language and with portions in the SAIL language, are presently available on the DEC-system 10 computer. However, these programs are readily adaptable to the larger desktop computers, and hence can be distributed widely to workers in endocrinology and other fields which employ protein fractionation and analysis. In particular, this will result in improved methods for measurement of the area under the peak, which in turn is utilized for construction of Scatchard plots in a variety of applications. This should improve our ability to detect factors which result in a minor shift in the position of the peak, and in better characterization of profiles wherein the peak of interest occurs as the "shoulder" on adjacent peak.

A new method has been developed for estimation of molecular weights on SDS-polyacrylamide gel electrophoresis when using pore-gradient gels. We have developed programs to compute the relationship between log(MW) and log(%T), where %T represents the gel concentration at the final position on the gel which the protein reaches at the end of the electrophoretic run. However, a theoretical study shows that this linear relationship is only an approximation. An exact physical-chemical model has been developed for analysis of this system, and has been implemented using non-linear least-squares curve fitting procedures. These methods ago: a linear relationship between log of distance migrated and log of duration of electrophoresis, for large t, and a linear relationship between log(MW) and log of distance migrated, for fixed t.

A new method has been developed for estimation of the calibration curve for sucrose density gradient sedimentation. By applying program

"CEPHAS" to the analysis of density gradient sedimentation, one can improve the precision of estimation of the position of the protein peaks, by approximately a factor of two. This improvement applies both to the position for the peak within a given tube, the variability between tubes within a given run of the ultracentrifuge, and the variability between runs. By virtue of computerization, variability is reduced by approximately a factor of two. This corresponds to approximately a two-fold improvement in the precision of the estimate of the sedimentation coefficient. Appropriate least-squares computer programs have been developed for estimation of sedimentation coefficient ("s") using the popular method of Martin and Ames. It has been demonstrated that the popular use of 2, 3, or 4, point to define the standard curve results in enormous 95% confidence limits for the "s" value for unknown proteins. When utilizing 2, 3, or 4 standard proteins, one can estimate the "relative sedimentation coefficient", i.e. the position of an unknown relative to a few arbitrarily selected standards. However one can not estimate the "s" value with precision, because there is the possibility that the standards may be nonrepresentative of the underlying population of all globular proteins.

Significance to Biomedical Research and the Program of the Institute:

The use of polyacrylamide gel electrophoresis, gel filtration, density gradient sedimentation, has been extremely popular in the analysis and characterization of protein and polypeptide hormones, the receptors for these hormones, and likewise the receptors for steroid hormones. Indeed these tools are indispensable. Accordingly, improved techniques for the calculation of the results, and the identification of the various factors describing the peak (e.g., skewness, kurtosis or shape) will result in improved extraction of information from prior experiments and improved design of future experiments. Further, these computer programs can save hours of technician time and therefore contribute to the efficiency of operation of the programs of the Institute. The identification of factors which affect band-width and hence resolution in polyacrydamide gel electrophoresis and isoelectric focusing is important in optimizing conditions for preparative PAGE. Further, this makes it possible to measure or at least to estimate the free diffusion coefficient for protein. In turn this permits estimation of molecular radius and derived parameters such as the fractional coefficient and axial ratio.

Proposed Course:

The project, involving both theoretical and experimental aspects, will continue in an attempt to develop more useful methods for characterization of protein and polypeptide hormones, and receptors for protein and steroid hormones. The use of continuous scanning of electrophoresis columns to permit estimation of the diffusion coefficient, molecular radius and microheterogeneity of size and/or charge is to continue, together with the corresponding theoretical studies necessary for the automated computerized analysis of results. The results of these studies will be applied to practical problems in the fractionation and preparation of peptide hormones and

receptors for peptide hormones. A mathematical theory of isoelectric focusing is under development.

Publications:

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Chrambach, A., and Rodbard, D., "Quantitative and Preparative Polyacrylamide Gel Electrophoresis"., in Hames, B.D. and Rickwood, D., (eds.), Gel Electrophoresis: A Practical Approach. Information Retrieval, London. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00043-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Mathematics and Statistics of Radioligand Assays

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
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	A. K. Thakur	Visiting Associate	ERRB, NICHD
Other	M. Jaffe	Bio Aid	ERRB, NICHD
	J. Huston	Programmer	DMB, DCRT

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Massachusetts General Hospital, Boston, Massachusetts

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NICHD, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mathematical and statistical theories of radioimmunoassay have been under progressive development, refinement, and testing. In the past year, attention has focused on immunoradiometric assays, two-site immunoradiometric (labeled antibody) assays (sandwich assays), enzyme-mediated immunoassay techniques (EMIT), enzyme-linked immunosorbent assays (ELISHA), and the labeled antibody membrane assay (LAMA). The chemical and mathematical equations describing these systems have been developed and used for computer simulation. Results provide a theoretical basis for the observed "paradoxical high dose hook effect" which occurs in many of these systems and suggests ways for improvement of these assay systems. The mathematical theory of optimization of RIA has been tested in practice and existing computer programs have been shown to be valid and useful guides to the experimentalist. Several new statistical methods for analysis of radioimmunoassay results have been developed. New computer programs have been developed for quality control of RIA, both within laboratories and for regional and world-wide collaborative studies.

Objectives:

Development of mathematical and statistical methods for evaluation of results in radioimmunoassay (RIA) systems for optimization of radioligand assays, and for statistical quality control of RIA laboratories.

Methods Employed:

Mathematical modeling, statistical analysis, computer stimulation and practical laboratory radioimmunoassays for a variety of hormones, including hCG, estradiol and human growth hormone.

Major Findings:

EMIT: The reaction scheme for EMIT has been formulated, the corresponding differential equations have been written, and these have been solved utilizing numerical techniques, employing the MLAB program of the DEC-System 10 computer at the National Institutes of Health, DCRT. This permits analysis of the individual factors which affect the assay, including the antibody concentration, labeled (enzyme tagged) antigen, incubation times, and the interaction of these factors. Further, we studied the effect of order of addition of the reagents, e.g., delayed addition of labeled antigen, delayed addition of substrate, etc. Also the roles of the 4 incubation times which describe the system have been studied in detail, both for systems in which antibody binding to ligand labeled enzyme results in a decrease in the Michaelis-Menten constant (Km), or in systems where the antibody-ligand-enzyme complex results in a decrease in the Vmax of an enzyme system. Computer programs have been developed to facilitate optimization either in terms of sensitivity, or in terms of precision at any desired target dose level.

Methods for calculation of the sensitivity (minimal detectable dose) of radioligand assays and related techniques have been developed and refined. Programs have been developed for the routine analysis of radioligand assay in the system. This permits refined testing for identity of standard and unknowns, not only in regard to slope, but also in regard to minimal and maximal responses, corresponding to the lower and upper asymptopes of the dose response curve. New programs have been developed for fitting the mass-action law, in the case of heterogeneity of receptor, or when multiple ligands combine simultaneously with multiple receptor sites. The statistical analyses of these and similar programs have been improved to permit refined testing of goodness of fit. Computer programs have also been adapted from the IBM 370 to the DEC-system 10, and in turn these programs have been simplified so that they can operate, in some cases on a hand-held calculator such as the Texas Instrument Model 59. In this manner, programs which once required relatively sophisticated computing facilities are now available to laboratories in developing countries throughout the world where such analyses were heretofore impossible. This adaption of software has been in collaboration with the program for quality control in reproduction research sponsored by the World Health Organization.

New methods have been developed for testing of parallelism of standard and unknown dose response curves, using a variety of rigorous statistical techniques. Several of these techniques are also readily adaptable to small desk-top or even hand-held calculators.

New programs have been developed for dose interpolation for radioimmunoassays. These involve use of splines, orthogonal polynomials, sums of hyperbolas constrained to be parallel everywhere and monotonic. These programs are useful applications as well. We have made further refinements in our programs for optimization of conditions for radioimmunoassay, two-site immunoradiometric assay, and enzyme labeled assays.

The characterization of the affinity of the antiserum is usually undertaken in the context of 1, 2, or 3 discreet classes of sites. We are now developing mathematical methods for characterization of these affinity profiles, in the context of a continuous distribution of affinities. This has required development of new computer programs, which should be generally useful to immunologists as well as to routine practitioners of radioimmunoassay.

New methods have been developed for comparison of results between two assay methods. Use of simple linear regression for this purpose if inappropriate and can be seriously misleading. One must take into account the errors in method A, the errors in method B, nonuniformity of these errors, and any interactions or correlations between these errors. The necessary computer programs have been developed for this type of analysis. This can be used for comparison of assay performance after a change of a simple reagent (e.g., and antiserum), for comparison of two different methods within a given laboratory, for comparison of the same mehtod or kit in two different laboratories, or comparison of different methods in different laboratories. This is an important tool for quality control. Several other new programs have been developed for RIA quality control. These provide the frequency distribution analysis of the slopes of samples within an assay, the frequency distribution of the counts recorded and frequency distribution of potency estimates for unknown samples within an assay.

Significance to Biomedical Research and the Program of the Institute:

This laboratory continues to be the major supplier of computer programs for analysis of radioimmunoassay results to the entire country, and to most of the world. In turn, dissemination of these programs results in "feedback" which enables us to revise and extend these programs so that they remain current and "state of the art." Further, numerous interesting applications become available, not only in the context of radioimmunoassay, but also in the context of radio-receptor assay and receptor systems in general. These programs provide automation, considerable saving of professional and technician time to members of the Reproduction Research Branch and National Institute of Child Health and Human Development, and provide estimates of

receptor assays and bioassays. As a result, experimental designs can be optimized with considerable saving in cost of reagents and professional time. Further, by making apparent the results of subtle improvements in the assay, these programs facilitate refinement of assays to stages which would otherwise not be possible.

Publications:

Rodbard, D.: Mathematics and Statistics of Radioimmunoassay and Radioligand Assays. In Syllabus, The Endocrine Society, Bethesda, Md. 1980.

Munson, P.J. Rodbard, D.: Statistical Methods for comparison of two Assay methods (weighted linear regression with errors in both variables). In Wilson, D.W. (Ed.): Quality Control of Radioimmunoassays, Tenovus Research Institute Symposium, 1980, in press.

Rodbard, D., Cole, B., and Munson, P.J.: The Need for Innovative Approaches to Radioimmunoassay Quality Control. In Wilson, D.W. (Ed.): Quality Control of Radioimmunoassays, Tenovus Research Institute Symposium, 1980, in press.

Davis, S.E., Jaffee, M.L., Munson, P.J., and Rodbard, D.: Radioimmunoassay data processing with a small programmable calculator. J. Immunoassay. 1 (1), 1980, 15-25.

Rodbard, D.: In Langan, J., Clapp, H. (Eds.):Mathematics and Statistics of Immuno Assay, Masson, New York, 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00146-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Structure and Function of Chorionic Gonadotropins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	H. C. Chen	Senior Investigator	ERRB, NICHD
Other	S. Matsuura	Visiting Scientist	ERRB, NICHD
	S. Kamatsuki	Visiting Associate	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

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NICHD, NIH, Bethesda, Maryland 20205

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3.0	3.0	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study chemical, immunological and biological properties of gonadotropins in tissues and biological fluids with emphasis on the isolation, purification, structure-function relationship and development of sensitive and specific detection methods to be applied in experimental and clinical endocrinology.

PHS-6040
(Rev. 10-76)

Objectives:

To isolate, purify and characterize the hCG-like substance (hCG') from urine and pituitary of normal subjects and to demonstrate its biological significances in reproduction and development.

To generate hCG-specific antisera based on chmical modification of polypeptides analogous to the unique carboxyl-terminal sequence of hCGβ subunit as antigens with attempt to obtain high affinity and high titer antibodies capable of specifically neutralizing hCG biological activity and adequate for the development of immunoassay methods in monitoring the gonadotropin action.

Methods Employed:

Solid phase peptide synthesis, amino acid analysis, chromatographic techniques (i.e., gel, ion exchange, partition and bioaffinity), rat uterine weight assay, immunization of rabbits, radioimmunoassay, radio-ligand receptor assay, interstitial cell testosterone production assay, high speed digital computer analysis, spectrophotometry and spectrofluorometry.

Major Findings:

1. The presence of hCG-like substance in pituitary gland.

A substance purified from human pituitary extracts by Sephadex G-100 gel chromatography followed by immunoaffinity chromatography using purified antibodies specific to the unique carboxyl-terminal peptide of hCGβ subunit has been shown to possess isoelectric points within the same range as a highly purified hCG (Lot CR119). The respective acidic components are both biologically active in stimulating mouse interstitial cells to produce testosterone and immunologically cross-reactive with hCG in a radioimmunoassay system that recognizes intact hCG but not its subunits. Additional data have indicated that an hCG' is present in human pituitary extracts which differs from LH in its physicochemical and immunological characteristics. It has been suggested that hCG' is ubiquitous. Since pituitary gland contains the highest amount among tissues of non-pregnant normal subjects, the presence of hCG' in the pituitary is in line with the notion that the hCG' may be a biosynthetic precursor of luteinizing hormone.

The content of hCG' as analyzed the acetone powder of human pituitary glands by an hCG-specific radioimmunoassay in the presence of a trypsin-inhibitor was found to be 1.12 μg hCG equivalent per gland in average. In collaboration with Dr. Anne Stockell Hartree of Cambridge University, we achieved 4500 folds purification of hCG' by a series of conventional chromatigraphy methods.

2. Production of hCG-specific antisera and determination of their antigenic stites.

Peptide analogues corresponding to the carboxyl-terminal peptide of hCG submit, namely (1) triacontapeptide (res. 116-145), (2) N^{α},N^{ε}-

dinitrophenyl-triacontapeptide, (3) desLys^{122}, Gly^{122}-pentatriacontapeptide (res. 111-145), (4) N$^{\alpha}$-pentaglycylpentatriacontapeptide (res. 111-145) have been chemically synthesized. Antisera were produced in rabbits against these peptides conjugated to bovine thyroglobulin.

Based on immunochemical interactions of these antibodies with peptides of various lengths and fragments representing different part of antigen molecules, three major antigenic sites have been identified. The first locus, Pro-Gln, corresponding to the terminal dipeptide is the primary recognition site and is essential for locking the second locus containing sequence, Arg-Leu-Pro-Gly (res. 133-136). The cooperative enhancement of these two loci appears to provide the maximal antigenicity and the basis of high specificity toward hCG without cross-reactivity to hLH. The third site resides at the amino-terminal portion consisting of Asp-Asp-Pro-Arg-Phe (res. 111-115) by itself is antigenic. However, this segment of peptide sequence as existed in hCG or hCGβ protein molecule is shown to be inaccessible for binding by these hCG-specific antisera. Thus, the low titer and binding affinity encountered by antisera against the carboxyl-terminal peptide as compared to anti-hCG and anti-hCGβ sera are attributable to the limitation of antigenicity existed in the sequence.

3. Development of an effective solvent system for eluting gonadotropins from concanavalin A affinity chromatography.

Elution of gonadotropins and other glycoproteins from concanavalin A has been previously performed with sugar derivatives. After a series of systematic studies using varaities of solvents and buffer solutions, we found that 1 M NH_4OH and other buffer solutions of equivalent basicity were more effective than the most widely used sugar derivative α-methyl-D-mannopyranoside. Brief treatment of gonadotropins/concanavalin A-Sepharose with either 1 M NH_4OH or 0.1 M Na_3PO_4 gave newly complete elution of gonadotropins from con A-Sepharose as revealed by protein contents, immunological, and biological activities. Advantages of 1 M NH_4OH are its simplicity, low cost, volatile nature and its ability to irreversibly inactivate concanavalin A.

This new system has been proven effective in extracting gonadotropins from urine of pregnant women, postmenopausal women, pregnant chimpanzee and orangntan. Based on immunological and biological assays, 86-97% of the active principles were recovered in one extraction, and 50-100 folds concentration and 2 to 6 folds increase in specific activities were achieved.

Significence to Biomedical Research

1. The presence of hCG-like substance in pituitary glands of normal subjects and its possible role as a biosynthetic precursor of hLH raises important questions as to the safety and value of fertility control by immunizing women with the hCGβ subunit on its unique carboxyl-terminal peptide.

2. Although distinguishing hCG from hLH is now possible, it should not attribute the mere presence of hCG in biological fluids to neoplasms.

3. Production of hCG-specific antisera based on synthetic peptides analogous to the unique carboxyl-terminal peptide of hCGβ subunit and understanding of their binding characteristics broaden the applicability and increase the reliability of immunoassay in detection of hCG and hCG-like substance in tissues and biological fluids.

4. A new simple and efficient solvent system to elute glycoproteins from an agglutinin enables us to concentrate sufficiently glycoprotein hormones in biological fluids and allows to devise practical hormone assay methods that increase substantially the specificity and sensibility.

Proposed Course

1. To conduct human pituitary tissue or cell culture investigate biosynthesis of hCG-like substance in relation to production of hLH.

2. To increase scale in isolation and purification of hCG-like substance from human pituitary extracts and to carry out structural studies in an attempt to understand homologues between hCG-like substance and hLH.

3. To continue production and purification of hCG-specific antisera against the synthetic peptides, and to prepare immunoabsorbent to be used for immunoassay and immunoaffinity chromatography for the purification of hCG-lik substance.

4. To collect, determine the content of hCG-like substance in tissues and biological fluids under physiological and pathophysiological conditions.

Publications

1. Matsuura, S., Ohashi, M., Chen, H. C., Shownkeen, R. C., Hartree, A. S., Reichert, L. E., Jr., Stevens, V. C., and Powell, J. E.: Physicochemical and immunological characterization of a human chorionic gonadotropin-like substance from human pituitary glands. Nature, In press, 1980.

2. Matsuura, S., and Chen, H. C.: A simple and effective solvent system for elution of gonadotropins from concanavalin A affinity chromatography. Analytical Biochemistry, In press, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00147-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Gonadotropins and of ACTH.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Maria L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
	Kevin J. Catt	Chief	ERRB, NICHD
Other	Keiko Hayashi	Visiting Fellow	ERRB, NICHD
	K. Nozu	Visiting Fellow	ERRB, NICHD
	J. R. Joshi	Visiting Fellow	ERRB, NICHD
	E. H. Charreau	Visiting Scientist	ERRB, NICHD
	J. Calvo	Guest Worker	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Sections on Molecular Endocrinology and Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad goal of this project is to understand the steps involved in the hormonal control of steroidogenesis. This includes the metabolic events that follow stimulation of trophic hormone receptors in the Leydig cells, luteal cell and adrenal fasciculata cells by gonadotropins and ACTH. Studies were focused on the interaction of hormones with their receptors, and on analysis of coupling mechanism between receptor occupancy and androgen, progesterone or corticosterone synthesis. The major topics examined were (1) the relationship of receptor occupancy to cyclic AMP and protein kinase activation during acute dose-related increases in steroid production and trophic actions of gonadotropin and ACTH; (2) characterization of cyclic AMP dependent protein kinases from steroidogenic tissues; (3) the sequence of events which leads to stimulation or inhibition of enzymes of the steroidogenic cells.

PHS-6040

Objectives.

To define the mechanisms of action of luteinizing hormone and ACTH upon steroidogenesis and growth in gonadal (rat Leydig cells) and murine Leydig tumor cells) and adrenal cells (fasciculata cells and Y1 cells).

Methods Employed.

Purification of Leydig cell by Metrizamide gradients. Characterization of LH, HDL, LDL and Prolactin receptors by binding analysis in cell membranes and intact Leydig cells. Measurements of extracellular, intracellular and receptor bound cyclic AMP by radioimmunoassay. Protein kinase assay. Gel filtration, sucrose gradient centrifugation, ion exchange chromatography, DEAE cellulose and polyacrylamide gel electrophoresis for characterization of protein kinase holoenzyme and regulatory subunits. Thin-layer chromatography on alumina and silica gel for characterization of steroids. Culture of Leydig cell tumors. Electron microscopy and light autoradiography. Polyacrylamide and SDS gel electrophoresis, isoelectric focusing and two-dimensional gel electrophoresis.

Major Findings:

1) Characterization and properties of adrenal fasciculata cell protein kinase, autophosphorylation and cAMP dependent endogenous phosphorylation.

The rat adrenal fasciculata cell contains two cAMP dependent protein kinase (PK) holoenzymes (H) with Mr of 160,000 (H1) and 106,000 (H2) that eluted as type I and II on DEAE cellulose chromatography. Dissociation by cAMP gave a 90,000 Mr regulatory subunit (R1) and a 10,000 Mr binding fragment (R2), derived from H1 and H2, respectively. Formation of the smaller component was reduced by protease inhibitors. Photoaffinity labeling of H1, H2 and R1 with 8-azido[^{32}P]cAMP and subsequent SDS PAGE revealed a predominant 56,000 Mr binding component; H1 also gave a minor 48,000 Mr species, and H2 and R1 gave a more abundant 42,000 Mr form. Limited proteolysis with thermolysin completely converted the 56,000 Mr form to the smaller species, predominatly the 42,000 Mr form. The properties of the two species of PK holoenzymes differed from those of type I and type II enzymes described in other tissues. neither of the enzymes were dissociated by high salt concentration, and histone caused partial dissociation of the H2 enzyme. MgATP was bound with high affinity by H1, H2, and R, but reduced the affinity for cAMP only in the H1 enzyme (by 10-fold). Only the H2 enzyme showed auto-phosphorylation, with predominant incorporation of ^{32}P into the 56,000 Mr subunit, and this was reduced in the presence of cAMP. Rapid cAMP-dependent phosphorylation by PK of protein substrate from fasciculata cell cytosol was observed in 13 protein bands with Mr range 30,000-130,000. The ED_{50} of cAMP-dependent phosphorylation was 10^{-7} M; for cGMP-induced phosphorylation of the same substrate the ED_{50} was 10^{-5}M. These results demonstrate the presence of two PK holoenzymes in FC and suggest that the R subunit of the smaller H2 undergoes rapid proteolysis. This can be partially inhibited by affinity labeling, giving an R subunit of 56,000 Mr for both H1 and H2. The two enzymes do not correspond in their properties to the type I and type II classes of PK. The marked difference in cAMP vs cGMP-

dependent phosphorylation of cell substrates gives further evidence that the steroidogenic response to exogenous cGMP is mediated through interaction of this nucleotide with the cyclic AMP binding sites.

2. Steroid biosynthetic lesions in gonadotropin-desensitized cells.

Administration of exogenous hCG or LHRH in adult male rats was followed by dose-dependent loss of testicular LH receptors and cAMP responses to hCG stimulation in vitro. Leydig cells from gonadotropin-treated animals with > 50% receptor loss showed reduced testosterone responses to hCG, dibutyryl cAMP, and choleragen, with concomitant accumulation of 17α-hydroxyprogesterone and sometimes progesterone (2,3). However, pregnenolone formation (measured in the presence of enzyme inhibitors) was normal or increased. These findings localized the major steroidogenic lesion to a partial block of the 17-20 desmolase and 17α-hydroxylase steps in androgen biosynthesis. The appearance of the transient steroidogenic defect before the marked drop in receptors, and its recovery before the receptors and cAMP responses returned to normal, suggested that the lesions were related to effects of LH on the Leydig cell other than the change in receptor sites. A role of E_2 in the enzymatic block produced in vivo by acute elevations of circulating gonadotropin was suggested by rapid increases of testicular E_2 followed by depletion of cytosolic receptors and conceomitant nuclear translocation of E_2 receptors. The inhibitory effect of endogenous E_2 on testicular steroidogenesis was prevented by Tamoxifen-induced depletion of cytoplasmic E_2 receptors. These findings indicate that the nuclear actions of testicular E_2 are responsible for the desmolase and 17α-hydroxylase lesions induced by hCG or LHRH. In addition to the E_2-dependent block in androgen synthesis, an earlier biosynthetic defect has been observed in Leydig cells from rats treated with high doses of hCG that caused almost complete loss of testicular LH receptors (2,13). This lesion was not caused by E_2 since it was not prevented by Tamoxifen, but may be related to the marked reduction of LH receptors and consequent lack of trophic stimulus to maintain early steroidogenic enzymes. The elucidation of steroid biosynthetic lesions produced during hormonal stimulation should contribute to the definition of intracellular control mechanisms that regulate the activity of steroidogenic enzymes.

3. Hormone-induced phosphorylation of endogenous proteins

Initial studies have demonstrated that both hCG and dibutyryl cyclic AMP induce a cascade of protein phosphorylation in the intact Leydig cell, and that these events are correlated with hormone-stimulated increases in cyclic AMP and preceded the consequent increases in testosterone production.

4. Lipoproteins and androgen biosynthesis.

Studies have been commenced on the role of lipoproteins in Leydig cell steroidogenesis. These include: a) Studies on the binding of high and low density lipoproteins to Leydig cell membranes b) Regulation and control of 3 hydroxy-3 methylglutaryl coenzyme A reductase (the rate-controlling enzyme in cholesterol biosynthesis) during the acute phase of hCG stimulation of androgen biosynthesis and during the desensitization phase affecting early steps of steroid biosynthesis.

Significance:

The hormonal control of Leydig cell function and androgen production is an essential component of male sexual development and reproductive capacity. In addition to its significance to the Endocrinology and Reproduction Research program, this project has the potential to provide knowledge about the general actions of peptide and protein hormones upon target cells in tissues other than the gonads.

Proposed Course.

1. (a) Investigation of the characteristics of protein kinase holoenzymes in adrenal and Leydig cell will be continued.

(b) The role of protein kinase in gonadotropin and ACTH action will be further analyzed by measurement of endogenous protein kinase activity and substrate phosphorylation in dispersed Leydig cells during LH stimulation in vitro.

(c) Investigation of the functional compartmentalization of cyclic AMP responses in the Leydig cell will be continued by localization of cyclic AMP bound to receptor protein and immunofluorescence microscopy of cyclic AMP in intact Leydig cells.

2. Activation of cholesterol side-chain cleavage and cholesterol esterase by LH in purified Leydig cells and of early enzymes prior to cholesterol formation.

3. The role of lipoproteins in Leydig cell steroidogenesis, and their participation during LH action, will be analyzed.

4. Studies on the effects of trophic hormones and steroid metabolites upon steroidogenic enzymes will be pursued.

Publications.

Dufau, M. L., Cigorraga, S., Baukal, A. J., Sorrell, S., Bator, J. M., Neubauer, J. F. and Catt, K. J.: Androgen biosynthesis in Leydig cells after testicular desensitization by luteinizing hormone-releasing hormone and human chorionic gonadotropin. Endocrinology 105, 1314-1320, 1980.

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Dufau, M. L., Cigorraga, S. B., Baukal, A. J., Baton, J. M., Sorrell, S.H., Neubauer, J. F. and Catt, K. J.: Steroid Biosynthetic lesions in gonadotropin-desensitized Leydig cells. Jour. Steroid Biochem. 11, 193-199, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00148-05

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Ontogeny of Gonadotropin Receptors and Gonadal Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt	Chief, ERRB	ERRB, NICHD
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Other	R. Clayton	Guest Worker	ERRB, NICHD
	I. Huhtaniemi	Visiting Scientist	ERRB, NICHD
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COOPERATING UNITS (if any)

None

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with analysis of developmental characteristics of gonadotropin receptors during fetal life and sexual maturation. Studies are performed on the emergence of receptors and biochemical responses of the pituitary-gonadal system during development, on the maturation of testicular and ovarian endocrine function, and on the control of gonadal sensitivity during sexual maturation.

PHS-6040

Major Findings:

(A) Pituitary GnRH Receptors and Gonadotropins During Development.

The functional role of GnRH receptors during development was evaluated by comparison with pituitary and serum gonadotropins and serum steroids during maturation and adult life. Pituitary GnRH receptors were assayed in pituitary homogenates by equilibration with ^{125}I-labeled ([D-Ser(tBu)^{6}]des-Gly^{10}GnRH N ethylamide), which binds to a single class of high-affinity GnRH receptors. In female rats, binding affinity was constant throughout life (K_a = $2x10^{9}M^{-1}$) and the concentration of sites varied with age. From the maximum value at day 20 (127 ± 14 fmol/mg protein), GnRH receptors decreased with age and at estrus/metestrus were 24 ± 7 fmol/mg at day 50 and 49 ± 9 fmol/mg at day 70. Over this period, GnRH receptors were correlated with pituitary LH content (r = 0.685), and serum LH levels decreased rapidly from 243 ± 13 ng/ml at day 15 to a plateau of 56 ± 3 ng/ml from day 25 to 70. In male rats, pituitary GnRH binding affinity ($2x10^{9}M^{-1}$) and the correlation between GnRH receptors and pituitary LH were similar to those in females. GnRH receptors were maximum at day 30 (87 ± 4 fmol/mg) and decreased gradually to a minimum (31 ± 3 fmol/mg) on day 60. There were similar changes in serum LH levels, while serum FSH increased from day 15 to day 30 (450 ± 18 ng/ml) and subsequently fell to a plateau on day 35 (334 ± 12 ng/ml). The changes in GnRH receptors during early development are consistent with the higher content of pituitary gonadotrophs in young rats, and with the greater responsiveness of the immature female pituitary to GnRH. The close correlation between GnRH binding, pituitary LH content, and serum gonadotropins demonstrates the physiological importance of the GnRH receptors during development.

(B) Ontogeny of Testicular Estradiol Receptors and Gonadal Responses.

The responsiveness of the immature testis to gonadotropins, and the occurrence of desensitization and receptor regulation, were analyzed in the neonatal rat testis in relationship to the appearance of estradiol receptors in the Leydig cell. The initial studies on these topics have indicated that estrogen receptors appear in the testis much earlier than previously reported, and that the immature testis has a quite different pattern of responsiveness than the adult tissue in terms of the relative trophic and desensitizing actions of LH or hCG upon gonadotropin receptors and androgen secretion.

Significance:

The characterization of pituitary and gonadal receptors and androgen regulation during fetal life and in immature animals will assist in clarification of mechanisms determining male phenotypic sexual development, and those controlling gonadal function during sexual maturation.

Proposed Course:

The role of protein kinase in mediating aspects of fetal testis maturation will be examined, and the development of pituitary function and GnRH receptors will be correlated with the profiles of gonadal receptors for LH/FSH and prolactin, and gonadal steroidogenesis during maturation in the rat.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00149-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Maria L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
	Kevin J. Catt	Chief	ERRB, NICHD
Other	Angela Solano	Visiting Fellow	ERRB, NICHD
	Alfonso Garcia-Vela	Guest Worker	ERRB, NICHD

COOPERATING UNITS (if any)

Dept. of Gynecology, Harvard Medical School, Mass. General Hospital, Boston, Mass.; Osaka Children's Hospital, Japan; Private Research Center, Atlanta, Georgia; Department of Medicine, University of Rome, Italy.

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Molecular Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Long range studies on the in vitro bioactivity of luteinizing hormone and chorionic gonadotropin in man and other species were continued. The recently developed in vitro bioassay for the measurement of LH and hCG in serum has been more extensively applied to studies on gonadotropin regulation in man and other species.

Methods Employed:

1. In vitro bioassay for serum gonadotropin based on testosterone production in collagenase-dispersed rat interstitial cells (RICT assay).

2. Radioimmunoassays for human LH. Radioimmunoassay for rat LH and for rLH subunits. Radioimmunoassay for rat prolactin, FSH and estradiol.

3. Radioreceptor assay for LH, prolactin and FSH using iodinated tracers of hCG, hGH and hFSH, respectively, to determine number of ovarian gonadotropin and prolactin receptor sites. Evaluation of hormone receptor occupancy in ovarian tissue was performed by elution of bound endogenous LH and subsequent measurement of the released hormone by RIA.

4. Gel filtration on Sephadex G-100 of rat pituitary extract, and measurement of eluted fractions by radioimmunoassay to detect intact rLH and subunits.

Major Findings:

1. In vitro Assay of LH in Rat Pituitary and Serum.

The rat was used as an animal model for analysis of the effects of steroids on LH bioactivity. This approach is convenient and advantageous in the investigation of the differing biopotency to immunopotency (B:I) ratios noted between cycling and males or postmenopausal females in studies on human subjects. Measurements of immunoactive and bioactive serum LH in the rat were performed by a recently optimized RIA and rat interstitial cell assay (RICT). Optimization of RIA was achieved by the use of ^{125}I-rLH tracer purified by Sepharose Concanavalin A, which gave ^{125}I-iodo-rLH with consistently high percentage binding to antiserum. In addition, extraction of rLH from serum was employed to improve the detection limit of the assay to an extent beyond the most sensitive available technique. The effective sensitivity of the RIA was increased 3- to 5-fold. This permitted assay of the biological samples at two or more dose levels with good precision. The sensitivity of the RICT bioassay was increased 4- to 5-fold by reduction of the incubation volume with adjustment of the cell number to gain sensitivity. The sensitivity of the modified bioassay was 3.1-6.25 ng RP-1, the sensitivity being increased 4- to 5-fold over the standard assay conditions used for primate serum. The sensitivity of the modified RICT assay was adequate for direct measurement of serum RLH without the use of an extraction step. The bioactive LH content of serum from adult male rats were 10-15 times lower than in men and 4-5 times lower than in the male rhesus monkey. Marked differences in B:I ratios were noted when the samples (control serum or pituitary and serum after LHRH stimulation were measured in terms of the RP-1 standard and in terms of the purified (Jutiz) rLH preparation, with reduction of B:I ratio from 2.7 to 1. These differences were attributable to the presence in the RP-1 standard of immunoactive material that is biologically inactive. After stimulation with LHRH, maximum increases in rLH bioactivity and immunoactivity were observed 15 min after subcutaneous injection, with a gradual decline to reach control values at 2 h. This contrasted with the more delayed and sustained responses observed

in men after s.c. LHRH administration, when bioactive and radioimmunoactive values reached maximum at 60 min and showed a slow return to control levels at 3 to 4 hours. In the male rat, as in men, there was no appreciable change in B:I ratio of LH from control levels of 1.21 ± 0.4, after LHRH administration. Comparative studies on the B:I ratio of the stored and circulating forms of LH in pituitary extracts and serum samples from normal and castrated male rats showed that the B:I ratio of serum LH was considerably higher (by 15-20%) than that of pituitary LH, and this difference was not changed by LHRH treatment. In contrast, after castration this situation was reversed, and pituitary LH exhibited a higher B:I ratio than the circulating hormone, though both were decreased compared to ratios in intact rats. Precise measurements of immunoactive and bioactive rLH in male rat plasma and B:I ratio can now be performed by these modified RICT and RIA procedures, and preferably should be based on highly purified hormone standards.

2. Modulation of Serum and Pituitary Luteinizing Hormone Bioactivity by Gonadal Steroids.

Comparison of the biological and immunological activities of circulating LH have shown marked differences between the bioactivity to immunoactivity of serum LH in cycling women, men, and postmenopausal women. These observations, and the demonstration of differences in FSH bioactivity in the rat and monkey, have suggested that gonadal steroids exert a modulatory effect on the intrinsic biological activity of gonadotropins. The investigation of this phenomenon would be facilitated by a suitable animal model to study the influence of gonadal steroids on the biological and immunological properties of both stored and secreted forms of LH. In this regard, we have found that the castrated male rat is a suitable animal model for these studies. Our findings have demonstrated that the B:I ratio of stored and secreted LH is significantly decreased between 5-25 days after castration (by 50%). The decrease in the B:I ratio of LH in castrated males was not due to increased subunit production and was prevented by administration of testosterone propionate from the time of castration. This demonstration of a decrease in the B:I ratio of LH after castration, and the previous observation of higher ratios in men than in premenopausal women, suggests that androgen or a conversion product modulate the biological activity of both stored and circulating LH molecules. Also, the effects of testosterone metabolites upon LH bioactivity were analyzed in castrated male rats treated with diethylstilbestrol or dihydrotestosterone. DHT not only prevented the drop of B:I ratio of serum LH observed in castration, but significantly increased the ratio above the control intact value (from 1 to 1.5). This treatment also prevented the fall of pituitary LH B:I ratio observed after castration. These results indicate that androgen per se modulates the bioactivity of both stored and released LH in the rat. In contrast, estrogen treatment only partially reversed the decrease in plasma B:I ratio. Estrogen, however, prevented the depletion of bio and radioimmunoactive LH from the pituitary usually observed from 5 to 10 days after castration and prevented the post-castration decrease in B:I ratio. In contrast to the findings with androgen treatment, where circulating radioimmunoactive LH was only marginally reduced and pituitary levels unchanged by the doses employed, DES treatment caused significant suppression of LH release from the pituitary into

the circulation. Thus, in this case the observed changes in B:I ratio could also be related to the decrease in secretion rate, as well as to the effect of estrogen per se. Thus, both androgen and estrogen modulates the biological activity of pituitary and plasma LH in the male rat.

In female rats, oophorectomy in diestrus has no effect on pituitary or serum LH B:I ratio. Oophorectomy during early proestrus abolishes the normal increase in B:I ratio of serum LH at the time of the preovulatory surge. The increased B:I ratio of plasma LH during the ovulatory surge appear to depend upon ovarian function, most probably estrogen secretion. Thus, the bioactivity of circulating LH appears to be rapidly modulated by gonadal steroid levels at the time of secretion.

3. Parallel Studies on LH Bioactivity and the Regulation of Ovarian Gonadotropin Receptors During the Rat Estrous Cycle.

These studies on the LH bioactivity and ovarian receptors have indicated that: a) The preovulatory LH surge is released from a pituitary pool of high biological activity. b) The LH surge is followed by a rapid decrease in ovarian LH and FSH receptors during the day of proestrus, and loss of these receptors was not due to occupancy by endogenous hormone. c) Up-regulation of prolactin receptors appears to occur at metestrus after the endogenous increase of serum prolactin at estrus. The concomitant increases in serum progesterone which occurred at the time of the increase of prolactin receptors could be related to prolactin action in the ovary. It is concluded from these studies that the negative regulation of gonadotropin receptors, and the positive regulation of prolactin receptors, are physiological components of the ovarian cycle.

4. Human Studies.

a) Comparative studies on serum LH bioactivity and radioimmunoactivity from birth to puberty in human serum are now in progress. A large number of samples of newborns are now being analyzed. These studies are in collaboration with Drs. T. Tsuruhara and K. Hayashi, Osaka Children's Hospital.

b) Studies on the pulsatile release of LH.

We are now evaluating the bioactivity and radioimmunoactivity of serum samples from normal males and cycling females, taken every 20 min for four hours. Preliminary observations are that bioactive LH showed a pulsatile release coincident with the radioimmunoactive measurements. However, the bioactivity of LH at the peak of the pulse is 2-4-fold larger than the corresponding immunoactive levels with consequent increase in bio-immunoratio. In contrast, in the female at all stages of the cycle, no pulsatile release of bioactive LH was observed as previously described for the radioimmunoactive levels. These studies were performed in collaboration with Dr. Franco Fraioli, University of Rome, and Drs. Janet McArthur and I. Beitins, Massachusetts General Hospital.

Significance to Biomedical Research:

The application of the RICT assay to the measurement of LH in human serum during LHRH stimulation has provided information about the qualitative and quantitative changes in the LH molecule at different stages of the menstrual cycle. Also, the application of the RICT assay to the assay of LH in monkey serum has provided the scientific community with a convenient method for the measurement of this hormone in primates. The recent development in the more sensitive RICT assay (5- to 10-fold) is of great value for the measurement of bioactive LH levels in children and in adults during suppression therapy. Also, this modification permitted measurement of the low circulating levels of LH in rats.

The RICT assay can be applied to different laboratories throughout the world, and is expected to give similar results when identical standards are employed. This is an improvement over certain aspects of RIA, which are subjected to the individual characteristics of each antibody employed for assay. The high sensitivity of the RICT assay will permit measurement of the levels of LH in serum from birth to puberty with good precision. This will also offer advantages over the RIA, which at these early times frequently measures levels just slightly above the lower limit of the sensitivity of the assay.

Proposed Course:

1. Studies on the modulation of rat LH bioactivity by steroids, and on the biosynthesis of LH in the pituitary, will be continued.

2. Studies on rat chorionic gonadotropin. We will extend our previous observations by following serum rCG biological activity throughout pregnancy from the time of fertilization. We will also evaluate rCG in extracts of blastocyst, implantation sites and placenta and will assay the bio-immunoactivity of the pituitary throughout pregnancy. Antibodies to rat LH, hCG, oLH, rabbit LH and subunits will be screened for cross-reactivity with rCG, for the purpose of establishing an immunofluorescence method to study CG distribution and secretion. Ultimately, when the site of secretion is identified, the hormone will be isolated, chemically characterized, and employed for production of antibodies to rCG.

3. Further investigation to gain insight into the nature of the B:I ratio differences observed between the different groups of subjects will include: a) examination of different LH pools released from the pituitary during stimulation studies; b) physical characterization of serum LH of the different groups of subjects.

4. Evaluation of LH values from birth through puberty.

5. Studies on the evaluation of bioactivity of LH during pulsatile release of the hormone will be continued.

Publications:

Solano, A. R., Garcia-Vela, A., Catt, K. J., and Dufau, M.: Modulation of Serum and Pituitary Luteinizing Hormone Bioactivity by Androgen in the Rat. Endocrinology 106: 1941-1948, 1980.

Blank, M. S., Dufau, M. L., and Friesen, H. G.: Demonstration of Potent, Gonadotropin-Like Biological Activity in the Serum of Rats During Mid-pregnancy. Life Sciences 25: 1023-1028, 1979.

Beitins, I. Z., Dufau, M. L., O'Laughlin, K., Bercu, B., McArthur, J. W., Crawford, J. D., and Catt, K. J.: Comparison of Biological and Immunological Activities in Serum LH in Normal Women During LHRH Infusion and Patients with Turner's Syndrome Treated with Estrogen. Symposium on LHRH, Memphis, Tennessee, December 9-10, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00150-05 RR

PERIOD COVERED
October 1, 1979 -September 30, 1980

TITLE OF PROJECT (80 characters or less)

Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other	J. Wimalasena	Visiting Fellow	ERRB, NICHD
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COOPERATING UNITS (if any)

None

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SECTION
Section on Molecular Endocrinology

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NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with solubilization, characterization and purification of gonadotropin and prolactin receptors and adenylate cyclase of testis and ovary. In addition to analyzing the properties of gonadotropin receptors and adenylate cyclase, these studies will lead to an analysis of the physical and functional relationships of the binding site and the enzyme complex in the cell membrane and in solution.

PHS-6040

Objectives.

(1) Solubilization of LH, FSH, and prolactin receptors from gonadal target cells; (2) Binding analysis of the solubilized preparations, comparative studies with particulate receptors; (3) Characterization of gonadotropin receptors; (4) Purification of LH/hCG receptors for structural analysis and antibody formation; (5) Characterization of particulate testicular and ovarian adenylate cyclase; (6) Solubilization of cyclase from testicular and ovarian membranes; and (7) Relationship between receptor sites and adenylate cyclase enzyme complex.

Methodology.

Assay of particulate and soluble receptors by ligand-binding and centrifugation, millipore filtration and polyethylene glycol precipitation; adenylate cyclase assay; cyclic AMP radioimmunoassay; sucrose density gradient centrifugation; gel filtration and affinity chromatography; SDS gel electrophoresis.

Major Findings.

1. Characterization and purification of LH/hCG receptors.

Ovarian LH/hCG receptors (R) were solubilized from membrane rich fractions by Triton X-100 or by low ionic strength buffer. Resolution of receptors by polyacrylamide gel electrophoresis (PAGE), combined with binding assay (by equilibration of R with ^{125}I-hCG directly in PAGE slices) showed molecular species with M_r of 165,000; 81,000; 48,000; 24,000 and 12,000. During chromatography on Sepharose-Concanavalin A, 80-90% of R was retained and could be eluted with methylmannoside 4 distinct peaks of R activity. PAGE analysis of each peak showed differences in the concentrations of the individual M_r species. For receptor purification, the lectin-gel step was followed by affinity chromatography on Sepharose 4B-hCG and elution of R by 0.025 M acetic acid. The affinity of the purified receptors (Ka) was 0.27×10^{10} M^{-1} and the binding capacity was 4200 pmol/mg, equivalent to 63% of the theoretical purity. PAGE analysis of the purified R showed identical molecular species as in the parent preparation, and Water-and detergent-soluble receptors showed similar physical characteristics. The molecular species characterized in this study indicate subunit structure with variable degrees of association.

2. LH induced nucleotide binding and activation of adenylate cyclase

The adenylate cyclase activity of Leydig cell homogenates and membrane fractions was found to be highly dependent on guanyl nucleotides. Enzyme responses to luteinizing hormone (LH) or human chorionic gonadotropin (HCG) were small in the absence of guanyl nucleotides. However, in the presence of 10^{-5} M Gpp(NH)p, both LH and hCG consistently stimulated testicular adenylate cyclase activity by up to 200%. Leydig cell membranes bound [^{3}H]Gpp(NH)p with high affinity (K_a = 1.5 X 10^7 M^{-1}) and binding capacity of 60 pmol/mg protein. During kinetic studies, the association rate constant

was 0.038 min^{-1}. In the presence of LH or hCG (10^{-11} to 10^{-8} M), concentration-dependent increases of 40 to 100% in Gpp(NH)p binding were observed in Leydig cell membranes. Kinetic studies showed that gonadotropin reduced the association rate constant to 0.73 x 10^6 M^{-1} min^{-1}, and the dissociation rate constant to 0.017 min^{-1}, with no effect on the quilibrium binding constant. The increase in Gpp(NH)p binding was not due to a change in receptor affinity, but was attributable to increased availability of nucleotide binding sites. The ED_{50} for adenylate cyclase activation by gonadotropin in the presence of Gpp(NH)p was identical with that observed for gonadotropin-induced binding of the GTP analog (5 x 10^{-8}MLH or hCG). These results indicate that gonadotropin-induced binding of Gpp(NH)p in Leydig cell membranes may represent interaction with the guanyl nucleotide regulatory site during hormonal activation of adenylate cyclase.

3) The binding characteristics of particulate and soluble rat ovarian prolactin receptors were studied and compared with those of receptors from the rabbit mammary gland. Initial observations have indicated that the ovary contains a large number of hindered prolactin receptors in the particulate fraction. These receptors were readily exposed by detergent treatment of the particulate sites, with a 3-fold increase in receptor binding capacity.

Proposed course.

1) Further purification and characterization of prolactin and LH/hCG receptors from rat ovarian homogenates.

2) Development of antibodies to purified receptor proteins, and their use in analyzing receptor function and turnover.

Significance.

Characterization of LH receptors and their relationships to adenylate cyclase will assist in understanding the basic biochemical mechanisms which control the reproductive functions of the testis and ovary. Also, further elucidation of the receptor-cyclase interaction will be of general value in understanding the mechanisms of action of protein hormones.

Publications:

Dufau, M. L., Hayashi, K, Nozu, K., Wimalasena, J., Sorrell, S., Baukal, A., and Catt, K. J.: Peptide hormone receptors and control of steroidogenesis. in Proceedings, Tenth International Congress of Endocrinology, Excerpta Medica, Amsterdam, 1980. In Press.

Dufau, M. L., Baukal, A. B., and Catt, K. J.: Hormone-induced guanyl nucleotide binding and activation of adenylate cyclase in the leydig cell. Proc. Natl. Acad. Sci., 1980. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00151-05 RR

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of Gonadotropin Receptors in Testis and Ovary

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt	Chief, ERRB	ERRB, NICHD
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Other	J. Harwood	Senior Staff Fellow	ERRB, NICHD
	R. Clayton	Visiting Scientist	ERRB, NICHD
	M. Katikineni	Research Associate	ERRB, NICHD
	T. Davies	Guest Worker (MRC Fellow)	
	V. Chan	Visiting Scientist	

COOPERATING UNITS (if any)

None

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This program is concerned with analysis of peptide hormone receptors in testis and ovary, with particular reference to the control of receptor concentration by changes in homologous and heterologous hormones. Desensitization of gonadal adenylate cyclase and steroidogenic responses by gonadotropic hormones is followed by receptor loss and turnover or processing of the hormone-receptor complex. These processes were investigated in Leydig cells and ovarian luteal cells to determine the effects of hormone-induced receptor regulation on gonadal responsiveness, and to analyze the mechanism and consequences of target-cell desensitization. The receptors and direct actions of gonadotropin-releasing hormone (GnRH) in the ovary and testis were also characterized, and analyzed in relation to the recently discovered anti-gonadal effects of GnRH agonists.

Objectives:

To analyze the regulation of gonadotropin receptor biosynthesis, expression and turnover in gonadal target cells.

Methods Employed:

Receptor concentration and affinity were measured by binding studies with ^{125}I-labeled LH, hCG, hFSH, hGH (for prolactin receptors) and GnRH, and [^{3}H]dihydroalprenolol for β-adrenergic receptors. Analysis of binding and GnRH agonist data was performed by computer methods applied to saturation curves and Scatchard plots. Luteinized ovaries were induced in immature female rats by treatment with PMSG and hCG. Isolated luteal and Leydig cells were prepared from ovary and testis, by collagenase dispersion and purification by density gradient centrifugation. Receptor binding (to LH, FSH, PRL, GnRH and β-receptors was measured in tissue homogenates and whole cells. Adenylate cyclase was measured in particulate ovarian fractions, and cyclic AMP and steroid production were determined in dispersed luteal and Leydig cells by radioimmunoassay.

Major Findings:

1. Regulation of testicular receptors

(a) Primate testicular LH receptors

The testicular luteinizing hormone (LH) receptors of the rhesus monkey and human have many features in common, including high equilibrium association constant, marked species specificity, and relatively low binding capacity. In adult male monkeys treated with 10,000 IU human chorionic gonadotropin, serum hCG was maximal on day 1 at 322 ±16 ng/ml and declined to 24.4±2.3 ng/ml by day 4. Serum testosterone was increased threefold during the subsequent 4 d (from 6.5±2.0 to 18.6±4.4 ng/ml) but serum progesterone remained unchanged. In contrast, serum 17α-hydroxyprogesterone increased 12-fold to 5.5±0.5 ng/ml at day 1 and was increased fourfold during the subsequent 3 d. The LH-receptor binding capacity of the primate testis was reduced by 18.3±6.0% on day 1, 51.7±7.4% on day 2, and 45.3±2.4% on day 4. Occupancy of the LH receptors by endogenously bound hCG was significant on day 1 but was negligible by day 4. These data demonstrate that gonadotropin-induced LH-receptor depletion occurs in the rhesus testis and indicate that primate gonadotropin receptors are susceptible to the regulatory processes recently described in the rat. In addition, the simultaneous and disproportionate accumulation of 17α-hydroxyprogesterone indicates that 17,20-desmolase was rate-limiting under these conditions in the primate testis Leydig cell.

(b) Effects of trophic hormone action on lactogen receptors in gonads and adrenal.

Receptor sites for lactogenic hormones such as prolactin (PRL), human growth hormone (hGH), and placental lactogens, are widely distributed in mammalian

tissues, including mammary glands, steroid-secreting cells of the adrenal, testis, and ovary, and target cells of steroid hormone action such as liver, prostrate, and kidney. Although the biological functions of lactogen binding sites remain uncertain, a relationship between prolactin and lipoprotein metabolism is implied by the occurrence of prolactin receptors in steroidogenic cells of the gonads and adrenal, and by the ability of prolactin to increase esterified cholesterol in the testis. Recently, loss of testicular prolactin receptors has been observed following elevation of circulating luteinizing hormone (LH) concentrations by gonadotropin releasing hormone (GnRH) and its agonist analogues. The hormone dependence of lactogen receptor sites in steroid-secreting cells was further analysed in rat testis, ovary, and adrenal glands after treatment with the respective trophic hormones, gonadotropin and ACTH. In each of these tissues, rapid and transient loss of lactogen receptors was observed after trophic hormone stimulation. These findings indicate that increased turnover of lactogen receptors is an important component of the target-cell response, and suggest that prolactin receptors might be involved in the transport of lipoprotein precursors for steroid biosynthesis.

(c) Regulation of testicular receptors by direct actions of GnRH.

Agonist analogs of gonadotropin-releasing hormone (GnRH) have been shown to exert antigonadal effects in male and female animals. In hypophysectomized male rats treated with follicle-stimulating hormone, administration of a potent GnRH agonist caused depletion of luteinizing hormone and prolactin receptors and marked suppression of serum testosterone levels. The possibility that such direct effects of GnRH agonists on testicular function could be expressed through specific receptors located in the interstitial cells of the testis was supported by the selective concentration of a ^{125}I-labeled GnRH agonist by the testis in vivo. Specific receptors for the releasing hormone were demonstrated in testis particles and dispersed interstitial cells by direct binding analysis with the ^{125}I-labled GnRH agonist. The binding affinity ($Ka=5x10^{9}M^{-1}$) and peptide specificity of the testicular GnRH binding sites were similar to those of anterior pituitary and ovarian GnRH receptors. The presence of GnRH receptors in the testis indicates that these sites mediate the direct inhibitory actions of GnRH agonists upon testicular endocrine function.

2. Regulation of ovarian receptors and responses.

(a) GnRH receptors and actions in ovarian luteal cells.

Gonadotropin-releasing hormone (GnRH) and its agonist analogs exert paradoxical antifertility effects in several species by causing regression of ovarian function. In collagenase-dispersed rat luteal cells, the agonist [D-Ser(tBu)^{6}]des-Gly^{10}-GnRH-N-ethylamide (GnRHa) caused inhibition of hCG- and epinephrine-stimulated progesterone production, but had no effect on the steroid dose-response curve to dibutyryl cAMP. The effect of the GnRH analog on the steroidogenic response to both hormones was to shift the dose-response curve to the right without changing the maximum production of progesterone.

Natural sequence GnRH also inhibited steroid production, with a half-maximal effect occurring at 5.10^{-9}M in agreement with the binding affinity of GnRH for its gonadal receptors ($Ka=5x10^{8}M^{-1}$). GnRHa also caused a shift to the right in the dose-response curve for cAMP production stimulated by hCG. However, no direct inhibitory effects of GnRH and its agonist analogs could be detected on hCG- or epinephrine-stimulated adenylate cyclase activity measured in ovarian homogenates. GnRH did not affect the binding affinity or capacity of rat ovarian receptors for hCG. Specific GnRH receptors in luteinized rat ovaries were identified with [^{125}I]GnRHa and found to have high affinity for the analog ($Ka=5x10^{9},M^{-1}$). A potent antagonist analog [D-pGlu^{1},D-Phe^{2},D-Trp^{3,6}]GnRH) was also bound with high affinity ($Ka = 3x10^{9}M^{-1}$) by the ovarian receptors.

These studies demonstrated that the ovary contains specific, high-affinity receptors for GnRH, through which GnRH and its agonist analogs can inhibit steroidogenic responses to hormonal stimulation. Since ther was no inhibition of dibutyryl cAMP-stimulated progesterone production and no direct action on hCG binding, it is suggested that GnRH interferes with the mechanism of hormonal stimulation of cAMP production.

(b) Regulation of ovarian GnRH receptors and effects on ovarian development.

The direct antagonistic actions of gonadotropin-releasing hormone (GnRH) and its agonist analogs on hormonal (hCG and epinephrine) activation of progesterone and cAMP production in isolated luteal cells are mediated by specific, high affinity GnRH receptors demonstrated in both ovarian membranes and isolated luteal cells. Administration of GnRH agonist analogs in vivo markedly inhibits PMS gonadotropin (PMSG)-induced follicular development and the increased formation of LH receptors after hCG-induced luteinization. Three GnRH agonist analogs with structural modifications at positions 6 and 10, given by osmotic minipump infusion, caused complete inhibition of the trophic actions of both gonadotropins. An antagonist analog which binds to the ovarian GnRH receptor with high affinity did not inhibit the gonadotropin-induced changes.

In the PMSG/hCG-primed immature rat, the effects of a single injection of GnRH were studied and compared to the action of hCG. Whereas the latter caused a marked loss of GnRH and LH receptors, GnRH caused a loss of ovarian LH receptors but a consistent increase in ovarian GnRH receptors; PRL receptors also showed a transient decline, and there was a fall in serum progesterone. In hypophysectomized rats, GnRH and hCG similarly caused a decrease in ovarian LH receptors and a transient change in the PRL receptor concentration. The GnRH-induced decrease in LH and PRL receptors in hypophysectomized animals indicates that GnRH analogs can act directly on the ovary, and that their effects are not only due to the release of gonadotropins from the pituitary gland. The direct inhibitory action of this class of GnRH peptides upon gonadal function constitutes an important component of the antifertility effects of these compounds.

Significance:

These findings extend knowledge of the regulation of receptor concentration and receptor-mediated processes in peptide hormone target tissues. The results are of general application to protein hormone receptors, and of particular relevance to the control of gonadal cell activities and reproductive function.

Proposed Course:

The mechanism by which homologous and heterologous hormones influence receptor turnover in the plasma membrane will be further investigated in the gonads. Combined biochemical and morphological studies on LH and PRL receptor regulation will be pursued in the Legdig cell. The biochemical basis for the inhibitory actions of GnRH agonists on gonadal function will be further analyzed in cultured granulosa and interstitial cells.

Publications:

Davies, T.F., Hodgen, G.D., Dufau, M.L., and Catt, K.J.: Regulation of primate testicular luteinizing hormone receptors and steroidogenesis. J. Clin. Invest., 64: 1070-1073, 1979.

Clayton, R.N., Harwood, J.P., and Catt, K.J.: Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. Nature, 282: 90-92, 1979.

Davies, T.F., Katikineni, M., Chan, V., Harwood, J.P., Dufau, M.L., and Catt, K.J.: Lactogenic receptor loss in hormone-stimulated steroidogenic cells. Nature, 283: 863-865, 1980.

Harwood, J. P., Richert, N. D., Dufau, M. L. and Catt, K. J.: Gonadotropin-induced desensitization of epinephrine action in the luteinized rat ovary. Endocrinology, 107: 280-287, 1980.

Harwood, J. P., Clayton, R. N. and Catt, K. J.: Ovarian gonadotropin-releasing hormone receptors. I. Properties and inhibition of luteal cell function. Endocrinology, 107: 407-412, 1980.

Harwood, J. P., Clayton, R. N., Chen, T. T., Knox, G., and Catt, K. J.: Ovarian gonadotropin-releasing hormone receptors. II. Regulation and effects on ovarian development. Endocrinology, 107: 414-421, 1980.

Clayton, R. N., Katikineni, M., Chan, V., Dufau, M. L., and Catt, K. J.: Direct inhibition of testicular function by gonadotropin-releasing hormone: Mediation by specific gonadotropin-releasing hormone receptors in interstitial cells. Proc. Nat. Acad. Sci. USA. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00152-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Characterization and Radioligand Assay of Gonadotropin Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt	Chief	ERRB, NICHD
	M. L. Dufau	Senior Investigator	ERRB, NICHD
Other	T. F. Davies	USPHS Fellow	ERRB, NICHD

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LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the development and use of analytical procedures for identification and characterization of gonadotropin receptor sites in the testis and ovary. Methods of gonadotropin labeling, characterization of the labeled tracer, and optimization of receptor assay methods, are evaluated and improved. Computerized methods for analysis of binding data from kinetic and equilibrium experiments, plus corrections for the tracer characteristics, are applied to studies on the properties of gonadotropin receptors. Native and modified gonadotropins are evaluated for receptor binding activity, and receptors are analyzed in animal and human tissues. Current studies include characterization of the human testicular receptors for LH and FSH, and analysis of receptor binding properties of highly purified glycoprotein hormones.

Objectives:

To characterize the binding properties of particulate gonadotropin receptors in gonadal cells, homogenates and membrane preparations. Also, to develop improved radioligand-receptor assays for LH/hCG and FSH, to apply such methods to assays of gonadotropin activity in vitro and structure-function studies on chemically modified gonadotropins, and to analyze the species specificity of gonadotropin receptors.

Methods Employed:

Quantitative binding studies with labeled gonadotropins. Radioligand-receptor assays of gonadotropins. Affinity chromatography upon receptor sites. Computer analysis and curve-fitting of receptor binding data. In vitro bioassay of LH in dispersed Leydig cells.

Major Findings:

1. Interaction of hCG with thyrotropin (TSH) receptors.

The TSH-competing activity of kaolin-acetone urine concentrates from patients with choriocarcinoma and/or thyroid dysfunction was investigated with a highly sensitive TSH radioligand-receptor assay using porcine thyroid homogenate and membrane-purified [^{125}I]bovine TSH. TSH-competing activity in urine concentrates from normal pregnant women and patients with hyperthyroid Graves' disease or primary and secondary hypothyroidism did not exceed that found in urinary concentrates from normal subjects. Euthyroid patients with choriocarcinoma, whose urinary hCG levels were similar to those found in pregnancy (2.3-73 µg/ml), had normal urinary TSH-competing activity. Increased urinary TSH-competing activity was detected in extracts from four choriocarcinoma patients with high urinary hCG levels (620-5,200 µg/ml). Three of these patients were biochemically hyperthyroid and their urine concentrates showed thyroid-stimulating activity in the mouse thyroid bioassay. Reduction in tumor activity after chemotherapy led to a concomitant reduction in urinary TSH-competing activity, hCG, and thyroid-stimulating activity. Both crude and purified hCG preparations obtained from pooled pregnancy urine exhibited TSH-competing activity in direct proportion to their hCG content, determined by radioreceptor assay. The crude and purified hCG also exhibited TSH-competing activity at concentrations (500-10,000 µg/ml hCG) similar to those found in urinary concentrates of the patients with choriocarcinoma. These data suggest that the TSH-competing activity in urinary concentrates from patients with metastatic choriocarcinoma is hCG itself, and that hCG acts as the abnormal thyroid stimulator in choriocarcinoma-associated hyperthyroidism by interacting with the thyroid cell membrane at or close to the TSH receptor site.

2. Binding properties of purified bovine LH and thyrotropin (TSH).

Highly purified preparations of bovine TSH (bTSH) and LH (bLH) and their subunits, obtained by affinity chromatography using immobilized antibodies directed against counterpart subunits, were assessed for biological activity

in radioligand-receptor assays for TSH and LH. After affinity purification against bLHβ, a TSH preparation whose initial potency in the LH assay had been 0.15% that of LH, failed to compete with [^{125}I]LH in amounts up to 100 µg. Thus, it appears that bTSH does not bind to LH receptors in the rat testis and that interaction of less purified TSH with gonadotropin receptors is attributable to LH contamination. In contrast, LH, whose initial potency in the TSH receptor assay was 0.6% that of TSH, retained a potency of 0.004% of TSH (equivalent to 3.6 mU/mg) after immunoadsorption by anti-bTSHβ. The retention of TSH receptor-binding activity by affinity-purified LH indicates that the LH molecule (like hCG) has a low intrinsic thyroid-stimulating activity. Affinity-purified LH subunits have little or no demonstrable affinity for the LH receptor in vitro. Affinity-purified TSH subunits and affinity-purified LH, however, exhibit very weak receptor-binding activity in the TSH radioligand-receptor assay. Evaluation of the capacity of the immunoadsorbents to remove TSH from artificial mixtures suggests that the residual binding does not result entirely from contamination, and therefore, that α-subunits as well as LH have some intrinsic TSH-binding activity.

Significance:

Radioligand-receptor systems continue to be of considerable value for hormone assay, and for structure-function studies on hormone molecules and receptor sites. The intrinsic TSH-like activities of hCG and LH can be attributed to structural similarities that permit binding of the gonadotropin molecules to the thyroid TSH receptors.

Proposed Course:

Studies on the temperature-dependence and thermodynamic properties of hormone-receptor interactions will be pursued. Also, radioligand-receptor assays will be compared with in vitro bioassay during analysis of the biological activities of native and modified gonadotropins. The basis of the species specificity of the primate LH receptor will be investigated, and characterization of the primate FSH receptor will be performed.

Publications:

Davies, T. F., Taliadouros, G. S., Catt, K. J., and Nisula, B. C.: Assessment of urinary thyrotropin-competing activity in choriocarcinoma and thyroid disease: Further evidence for human chorionic gonadotropin interacting at the thyroid cell membrane. J. Clin. Endocrinol. Metab. 49: 353-357, 1979.

Williams, J. F., Davies, T. F., Catt, K. J., and Pierce, J. G.: Receptor-binding activity of highly purified bovine luteinizing hormone and thyrotropin, and their subunits. Endocrinology 106: 1353-1359, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00160-05 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biology of the Adrenocortical Cell: Molecular Events in Steroid Biosynthesis and Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	ERRB, NICHD
Other	Alan Goff	Visiting Fellow	ERRB, NICHD
	Kenji Fujieda	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	3	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-term aim of this project is to explore in greater depth, the biology of the steroid-synthesizing cell. There are several areas of uncertainly as to the molecular events involving 1) initiation, propagaton, and termination of steroid synthesis, 2) intercompartmental movement of steroid precursors and intermediates, 3) secretion of steroid products. Other areas of uncertainly involve 1) the interaction of subcellular organelles, e.g., mitochondria, endoplasmic reticulum, Golgi apparata, microtubules, microfilaments, lipid droplets, etc. 2) cell-cell interaction or zone-zone interaction (different cells are programmed to secrete predominatly one type of steroid, i.e., glucocorticoid, mineralocorticoid, and sex steroids), 3) life-span and turnover of adrenocorlical cells. Current areas of research involving: 1) identification, characterization, and physiology of noncatalytic proteins (membrane bound and/or soluble) of the adrenal cortex that interact with specific steroid ligands; 2) examination of the role of microtubules and contractile proteins in steroid synthesis; 3) identification, characterization and physiology of adrenocortical secretory proteins; 4) examination of soluble stimulatory and inhibitory factors.

PHS-6040

Project Description

I. Non-catalytic steroid-binding proteins.

A. Rationale: The rate-limiting step in steroid synthesis in considered to be the conversion of cholesterol to pregnenolone (i.e., the cholesterol lyase reaction). This reaction occurs at an intramitochondrial site (possibly associated with the outer membrane). Cholesterol, which is an absolute requirement for steroid synthesis, is a ubiquitous sterol, being found in membranes and stored esterified to fatty acids in cytoplasmic lipid droplets; it can also be made available through de novo synthesis and extraction from the circulation. The exact location and pool size of cholesterol utilized in steroid synthesis are not known. It is presumed, however, that the principal source of cholesterol is extramitochondrial. If this is true, then there must be a mechanism for transporting cholesterol into the mitochondrion where the cholesterol lyase is located. The molecular events involved in this transport are not known. Similarly, pregnenolone, the product of the rate-limiting step must move to specific extramitochondrial sites for further enzymatic alterations leading to the formation of cortisol, aldosterone, or one of the sex steroids. The molecular events involved in this transport are also not known. It is conceivable that specific proteins acting as transport vehicles are involved.

B. Major Findings: Using standard techniques of subcellular fractionation and methods of protein separation, six specific proteins have been identified to date.

1. Cholesterol/cholesterol sulfate-binding protein No. 1. This is a heat stable protein which has been partially characterized. The initial characterization studies have been published in the Journal of Steroid Biochemistry, 9:721-730, 1978.

2. Cholesterol/cholesterol sulfate-binding protein No. 2 This is a heat sensitive protein whose initial characterization has been published in the Journal of Steroid Biochemistry, 13:73-82, 1980. Purification of these two proteins is now being undertaken.

3. 20 α-hydroxycholesterol-binding protein. This project remains undeveloped.

4. Pregnenolone-binding protein. This is a heat labile protein whose initial characterization has been published in the Journal of Biological Chemistry, 252: 464-470, 1977. Purification of this protein is currently being completed.

5. Pregnenolone sulfate-binding protein. This is a heat labile protein (clearly distinct from the pregnenolone-binding protein) whose initial characterization was published in Biochemistry, 17: 4557-4563, 1978. Purification of this protein is in progress.

6. 21-hydroxypregnenolone-binding protein. This is a heat labile protein whose initial characterization is being prepared for publication.

C. Significance: The physiological importance of these findings remains to determined. From a theoretical point of view, however, they are quite interesting. It is well recognized that labile and stable protein factors play a role in control and regulation of steroid synthesis but to date, no specific proteins have been described. Our studies represent the first such undertaking of this kind. Much needs to be done yet, but given the fact that heat-labile and heat activated proteins which bind specifically cholesterol and cholesterol sulfate, substrates for the side-chain cleavage enzyme system, and heat-labile proteins which bind specifically pregnenolone and pregnenolone sulfate, products of the side-chain cleavage enzyme system, and the fact that the cleavage enzyme system (cholesterol lyase) is considered the rate-limiting step in steroid synthesis, these findings are of potential importance. In addition, there may be other noncatalytic proteins involved in other steroid enzyme systems of the adrenal cortex which when absent could be pathologically related to certain well-recognized enzyme deficiency diseases of the adrenal cortex, viz, congenital adrenal hyperplasia.

D. Current and Future Plans: (1) To continue characterizing the proteins identified to date. (2) To continue our search for other non-catalytic proteins. (3) To complete purification of these proteins using a variety of techniques. (4) To use highly purified preparations to a) examine intrinsic enzymatic activity, and b) to prepare antibodies for immunological studies. (5) To reconstitute the cholesterol lyase system and other steroid mixed function oxidase systems. (6) To examine the subcellular localization of these proteins by tagging experiments. (7) To examine regulation of their synthesis and metabolism.

II. Microtubules.

A. Rationale: Enzyme systems involved in steroid biosynthesis are compartmentalized (mitochondrial, microsomal,? soluble) but nothing is known about the molecular mechanisms of transfer of steroids from compartment to compartment nor is anything known about the molecular mechanisms involved in steroid secretion. It has been proposed that microtubules or microfilaments may function in this capacity and thus studies have been undertaken to examine this possibility.

B. Major Findings: (1) Our initial efforts were to establish methods for qualitative analysis. A study has been completed comparing colchicane-binding activity (tubulin) in the adrenal cortex of dog, monkey, pig, guinea pig and cow, and brain of the pig and guinea pig. In addition, electron microscopic studies of adrenocortical microtubules have been made. A portion of these studies have been published in Biochimica et Biophysica Acta 495: 119, 1977. (2) There is some inconsistency in the literature concerning the effect of antimicrotubular drugs on adrenal steroidogenesis.

One report indicated that these drugs stimulated steroidogenesis in a mouse tumor cell line, while another report found no such stimulation using normal rat adrenocortical cells in culture. We also examined this question using isolated adrenocortical cells obtained from normal adult male rats. We found that a variety of antimicrotubular drugs will stimulate steroid synthesis in a dose-dependent fashion with the maximal response being equivalent to that obtained with either ACTH or cAMP. The mechanism of this response remains to be elucidated. These results have been published in Endocrinology, 103: 1281-1288, 1978. (3) A study was published in BBA, 581: 79-86, 1979, comparing bovine brain and adrenal cortex (neural versus non-neural) microtubules purified through three-four cycles of assembly/disassembly. The comparison included (a) colchicine binding activity, (b) tyrosylation using a specific tubulin-tyrosine ligase (which adds a tyrosine residue to the C-terminal glutamate or glutamine of the α chain) in conjunction with carboxy-peptidase A (which recovers the tyrosine), (c) examination of so-called microtubule associated proteins on SDS-PAGE, and (d) percent protein and amino acid analysis using the auto-analyzer.

C. Significance: The role of microtubules in a variety of cell function (e.g., mitosis, motility, cell shape, etc.) has been well-delineated. The microtubule organelle is found in all cells, although its highest concentration appears to be in neural tissue. How this ubiquitous organelle which plays a role in multiple cell activities is involved in such a higly specialized function as steroidogenesis is simply not known. This remains an interesting question in cell biology.

D. Future Plans: Elucidation of the microtubular and microfilamentation systems of the adrenal cortex in regulation of steroidogenesis currently suffers from a total lack of careful morphological correlative studies. Our plans call for the establishment of stable, homogenous population of adrenocortical cells in tissue culture. With such a culture system, we plan to perform a variety of morphological studies utilizing transmission electromicroscopy, scanning electromicroscopy, and immuno-fluorescent or ferritin tagging techniques.

III. Steroid Secretory Proteins. These studies have not progressed very far as yet but they are based on the theoretical consideration that steroids are released from specific cellular proteins during the secretory process.

IV. Stimulation of steroidogenesis by a soluble adrenocortical factor(s).

What has progressed to some extent has been the examination of a soluble stimulatory factor(s) detected in the adrenal cortex following the in vivo administration of ACTH to hypophysectomized rats. This heat-sensitive factor will cause a 3-4 fold increase in the rate of pregnenolone formation using partially purified mitochondria obtained from hypophysectomized untreated litter mates. The stimulation is dose-dependent, and cytosol prepared from non-adrenal tissue does not contain stimulating activity. Partial characterization of this activity has been submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00164-04 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Strategy and Mechanisms of Fractionation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	N. Y. Nguyen	Visiting Scientist	ERRB, NICHD
Other	A. Chrambach	Senior Investigator	ERRB, NICHD
	L. Hjelmeland	Staff Fellow	DPB, NICHD
	B. An der Lan	Visisting Fellow	DBP, FDA

COOPERATING UNITS (if any)

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Endocrinology and Reproduction Research Branch

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. New electrophoretic approaches, applicable to separation of cells and subcellular elements, were introduced.

2. Fractionation of biological macromolecules by electrofusing was found to become increasingly flexible.

PHS-6040

1. Separation of cells and subcellular elements (in collaboration with A. Chrambach and L. M. Hjelmeland): Requirements for detergents in the separation of cells and subcellular elements makes one expect denaturation of protein with loss of biological activities. With the development of non-denaturating amphoteric detergents, it appears possible to recover active proteins.

2. Protein separation by electrofocusing has become increasingly flexible (in collaboration with A. Chrambach, L. M. Hjelmeland and B. An der Lan): pH gradient engineering in application to a particular fractionation problem, is accomplished systematically by proper choice of anolyte, catholyte, constituent mixture and field strength.

Publications:

Chrambach, A., Nguyen, N. Y., and Hjelmeland, L. M.: New Electrophoretic Approaches Applicable to Cell Separation. In Peeters, H. (Ed.): Separation of Cells and Subcellular Elements. Oxford, New York, Pergamon Press, 1979, pp. 31-37.

Chrambach, A., Hjelmeland, L. M., Nguyen, N. Y., and An der Lan, B.: Gel Electrofocusing with Increased Degrees of Freedom. In Radola, B. J. (Ed.): Electrophoresis '79. Berlin, New York, Walter de Gruyter, 1980, pp. 3-22.

Nguyen, N. Y., and Chrambach, A.: Analytical and Preparative Gel Isotachophoresis of Macromolecules. In Hames, B. D., and Rickwood, D. (Eds.): Gel Electrophoresis: A Practical Approach. London, Information Retrieval, Ltd., 1980, in press.

Chrambach, A., and Rodbard, D.: Quantitative and Preparative Polyacrylamide Gel Electrophoresis. In Hames, B. D., and Rickwood, D. (Eds.): Gel Electrophoresis: A Practical Approach. London, Information Retrieval, Ltd., 1980, in press.

An der Lan, B., and Chrambach, A.: Analytical and Preparative Gel Electrofocusing. In Hames, B. D., and Rickwood, D. (Eds.): Gel Electrophoresis: A Practical Approach. London, Information Retrieval, Ltd., 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00165-05 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Isolation and Characterization of Protein Hormones and Other Active Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	A. Chrambach	Senior Investigator	ERRB, NICHD
Other	S. Allenmark	Guest Scientist	Linkoping University[1]
	E. Leonard	Senior Investigator	NCI
	K. Felgenhauer	Professor	University of Koln[2]
	G. Kapadia	Biochemist	Meloy Labs[3]

COOPERATING UNITS (if any)
[1]Biochemistry Dept., Linkoping University, Linkoping, Sweden;
[2]Nervenklinik, University of Koln Medical School, Koln, GFR;
[3]Springfield, Va.

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) A procedure for the preparation of serum macrophage stimulating factor by Sephadex gel electrofocusing was developed. 2) The nature of the heterogeneity of immunoglobulins in electrofocusing (EF) was elucidated. This provides the basis for an improved clinical analysis for oligoclonal antibodies by gel electrophoretic methods. 3) hCG of high specific activity was isolated from commercial material in a single-step procedure, using re-stacking.

PHS-6040

1) Preparation of serum macrophage stimulating factor (MSF) (in collaboration with S. Allenmark and E. Leonard):

Objectives: To characterize and isolate a serum activity capable of stimulating macrophage production.

Methods employed: To circumvent a previous 4-5 step isolation procedure with correspondingly low yield we intended to utilize the previous finding that the activity exhibited a pI of 7, higher than that of most proteins, in flat-bed EF. It was found, however, that the MSF activity under conditions of improved heat dissipation at 0-5C has a pI of 5, and that either during a chromatographic step, or in flat-bed EF, this is transformed to 7. This transformation was utilized in the design of a 2-step isolation procedure.

Major findings: Through temperature control at 0-5C, MSF with pI 5 can be prepared by EF on cylindrical gels. At R.T. the excised protein can be transformed to one with pI 7 and isolated free from other proteins (which do not undergo the specific presumably enzymatic transformation) by gel EF.

Significance to Biomedical Research: Isolation of MSF provides the basis for metabolic, clinical and biochemical studies of the new activity.

Proposed course: Exploitation of the procedure to the preparation of milligram amounts of MSF.

2) EF of immunoglobulins (IgG) (in collaboration with K. Felgenhauer):

Objectives: To spread the gel pattern of IgG across the entire migration path in gel EF and/or gel electrophoresis, in order to augment detection sensitivity for monoclonal antibodies beyond that obtainable today on a mere 10-15% of the migration path. In addition, the apparent artifact in EF by which IgG produces 20-30 distinct zones rather than the continuum expected from thousands of charge isomeric species needed to be elucidated and avoided, before EF could be safely applied to clinical analysis of monoclonal IgG.

Methods Employed: The problem was simplified by preparation of 26 groups of charge isomeric IgG species, using isotachophoresis on polyacrylamide gel (ITPPA). The Ferguson plots of these in PAGE revealed a great number of oligomeric species. EF in the required pI-range of 6-10 was stabilized by appropriate selection of conditions to allow for prolonged EF times. These in turn allowed us to conduct EF for sufficient time to obtain a coalescence of all oligomeric forms at the pIs of the monomers. This coalescence abolished the artifactual zone phenomenon in EF. In addition, optimization of pH in PAGE for the basic IgG species and optimization of stacking limits provided for PAGE patterns of IgG occupying the entire gel length.

Major findings: The relatively acidic IgG components aggregate upon isolation from serum. The aggregates appear in EF under conventional conditions of agarose or polyacrylamide gel EF as artifactual zones. Prolonged

EF conditions obtainable at present only on polyacrylamide cylinders abolish this artifact by providing sufficient time for aggregate coalescence with the monomers at their pIs. In addition, appropriate choice of PAGE conditions extends the IgG pattern to the entire gel length in electrophoresis.

Significance to Biomedical Research: IgG patterns in both PAGE and gel EF which are continuous and which cover the entire gel length provide clinical analysis with an artifact-free and magnified view of IgG species, their variation in specific diseases (e.g. viral brain infections giving rise to compartmentalized monoclonal IgG), and allow one to pinpoint specific IgG components with increased detection sensitivity.

Proposed course: Translation of the optimized EF and PAGE conditions to agarose slab techniques, capable of 50-10 clinical analyses per day and simpler than the research techniques which have been applied to date in the above-cited study.

3) Isolation of hCG with high specific bioactivity by a simplified method (in collaboration with G. Kapadia):

Objective: Preparation of milligram amounts of hCG from crude starting material, using a maximally simplified method.

Methods: Elution PAGE was applied under conditions where contaminants migrated in the stack, while hCG unstacked. To be able to elute hCG in concentrated form, the unstacked hCG (5-6 isohormone forms separated under those conditions) was re-stacked, taking advantage of a hitherto not exploited feature of the multiphasic zone electrophoresis theory of Jovin. This allowed for elution of hCG in a single concentrated zone (a second stack) (see Z01 HD 00171-04 RR).

Major findings: Milligram amounts of hCG with 8-12,000 IU/mg were prepared in a single elution-PAGE step from crude commercial starting material with 3,000 IU/mg. The yield was 20% on a weight basis.

Significance to Biochemical Research: Provision of an easy, one-step procedure for preparing milligram amounts of hCG is an aid for biochemical studies of the molecule.

Proposed course: No further work on this problem is intended.

Publications:

Ben-David, M., and Chrambach, A.: A Method for Isolation by Gel Electrofocusing of Isohormones B and C of Human Prolactin from Amniotic Fluid. J. Endocrinology 84: 125-133, 1980.

Lang, U., Kahn, C. R., and Chrambach, A.: Characterization of the Insulin Receptor and Insulin Degrading Activity from Human Lymphocytes by Quantituantitative Polyacrylamide Gel Electrophoresis. Endocrinology 106:40-49, 1980.

Ben-Or, S., and Chrambach, A.: Multiple Forms of Glucocorticosteroid Receptors in the Neural Retina of the Chick Embryo, Revealed by Polyacrylamide Gel Electrophoresis. Archives Biochem. Biophys., 1980, in press.

Nguyen, N. Y., and Chrambach, A.: A 3-Step Method for Isolating a Few to Several Hundred Milligrams of Protein. J. Biochem. Biophys. Methods 1: 171-187, 1979.

An der Lan, B., Habig, W. H., Hardegree, M. D., and Chrambach, A.: Heterogeneity of ^{125}I-Tetanus Toxin in IFPA and PAGE. Archives Biochem. Biophys. 200: 206-215, 1980.

Nwokoro, N., Chen, H.-C., and Chrambach, A.: Physical, Biological and Immunological Characterization of Highly Purified Urinary Human Chorionic Gonadotropin Components Separated by Gel Electrofocusing. Endocrinology, 1980, in press.

Kapadia, G., Vaitukaitis, J., and Chrambach, A.: One-Step Isolation of Human Chorionic Gonadotropin in Milligram Amounts, Using Selective Steady-State Stacking on Polyacrylamide Gel. Preparative Biochem., 1980, in press.

Houghten, R., and Chrambach, A.: Studies on the Reaction Between Human Growth Hormone and Oleic Acid, Using Polyacrylamide Gel Electrophoresis. Archives Biochem. Biophys. 197: 163-169, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00170-04 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Randomized Study for the Treatment of Chromophobe Adenomas of the Pituitary Gland with Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	NICHD, ERRB
Other	C. Ronald Kahn	Senior Investigator	NIAMDD, DB
	Jesse Roth	Chief	NIAMDD, DB

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since the natural history of chromophobe adenomas unassociated with visual impairment is uncertain, the approach to therapy in patients with this disorder is controversial. Complications often attributed to tumor growth may, in fact, be sequalae of the treatment administered, whether it be radiotherapy or surgery. The purpose of this project is two-fold: 1) to observe the natural history of untreated chromophobe adenomas in patients who do not present initially with visual disturbances and 2) to compare their clinical course with that of patients treated by one commonly employed modality, i.e., radiation therapy. Thus, patients will be randomized into two groups: one group will receive no therapy and one group will be treated with conventional pituitary irradiation. In a sense, each group is serving as a control for the other group. Over the study period specific clinical and laboratory abnormalities will be assessed in each group and compared in an attempt to help guide physicians in the treatment of this disorder.

PHS-6040

Project Description:

Male and female patient less than 65 who have carried the diagnosis of a pituitary tumor for less than one year and for which they have not received prior treatment are eligible. Patients whith acromegaly and Cushing's Syndrome will not be studied. Minors will be admitted to the protocol only with their own and parental consent. If a defect in visual fields using a one millimeter white object is found the patient will receive treatment in a standard fashion and will not be considered for this protocol. In addition, patients with the following conditions will be excluded from the protocol: 1) severe, incapacitating headaches; 2) complete erosion of the inferior margin (floor) of the sella turcica; 3) clinical evidence of cavernous sinus involvement (e.g., painful opthalmoplegia).

Historically, conventional radiation therapy has played a major role in the management of chromophobe adenomas of the pituitary. Complications such as optic neuritis, extraocular muscle paralysis and loss of memory which might be attributable to therapy are rare. When further endocrine hypofunction follows pituitary radiation, the relative contributions of the radiation and the underlying adenoma itself cannot be separated. This question could be answered in this prospective study of treated and untreated patients.

The purpose of this project is then to observe the natural history of untreated chromophobe adenomas which have not initially produced a visual field defect and to compare their clinical course with that of patients receiving conventional pituitary irradiation. Through a comparison of the long-term clinical and laboratory follow-up of these patient populations, a meaningful analysis of the relative impact of pituitary irradiation on the natural history of this disorder when left untreated could be made. In addition, specific clinical and laboratory adnormalities which will guide the physician in his treatment of these patients may be identified.

Major Findings:

This project has not been in existence very long, and since it will eventually involve a large number of patients followed for at least ten years, no conclusions can be made as yet. To date, four (4) patients have been randomized.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00171-04 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Other PAGE Instrumentation and Procedures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	A. Chrambach	Senior Investigator	ERRB, NICHD
Other	N. Y. Nguyen	Visiting Scientist	ERRB, NICHD
	B. An der Lan	Visiting Fellow	DBP, FDA
	L. Hjelmeland	Staff Fellow	DPB, NICHD
	G. Kapadia	Biochemist	Meloy Laboratories[1]
	J. Sullivan	Engineer	DRS, BEIB

COOPERATING UNITS (if any)

[1]Meloy Laboratories, Springfield, Va.

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) Detailed laboratory procedures for isotachophoresis, "quantitative" PAGE and electrofocusing on polyacrylamide gel were written; 2) An apparatus for packing of multiple 6 and 18 mm Sephadex electrofocusing gel tubes was designed and constructed; 3) A zwitterionic monomer was synthesized, polymerized to a cross-linked gel and tested for its capacity of bridging conductance gaps in EF and its effect on protein mobilities; 4) Restacking of unstacked protein zones, postulated by the Jovin theory of multiphasic buffer systems, was demonstrated for the first time and applied to the simultaneous isolation of several hCG isohormones; 5) Purity of protein isolation by Steady-State Stacking was greatly improved by replacing polyacrylamide with agarose in the extraction and concentration step.

PHS-6040

Project Description

1) Procedures of Gel Electrophoresis, Electrofocusing and Isotachophoresis (in collaboration with D. Rodbard, N. Y. Nguyen and B. An der Lan):

Objectives: To summarize up-to-date detailed procedures of electrophoretic protein fractionation for the biochemical user.

Significance: Previously we have published summaries on the strategy (J. Mol. and Cell. Biochem. 29: 23, 1980) and on the instrumentation (Methods of Prot. Separation, Vol. II, p. 27, 1976) of gel electrophoretic protein fractionation The three chapters on procedure written during this year (ref. 1-3) complement these previous ones. Together, they represent a "pre-monograph" on every major aspect of gel electrophoretic separation methods.

Proposed Course: It is planned to unify the presentation of the above cited three aspects of fractionation in a monograph, to provide the biochemical user with a more readily accessible research tool.

2) Sephadex gel tube apparatus for electrofocusing (in collaboration with B. An der Lan and J. Sullivan):

Objectives: In dealing with very large molecules, in excess of 10^6 MW (e.g. some forms of Tetanus toxin), or with very temperature-sensitive proteins [e.g. Macrophage Stimulating Factor (MSF)] we needed an electrofocusing (EF) method on a matrix with larger average pore size than "non-restrictive" types of highly cross-linked polyacrylamide.

Methods Employed: The good heat transfer characteristics of tube gels and the non-restrictiveness of Sephadex were combined, and practical multi-tube apparatus for making cylindrical Sephadex gels was designed and constructed.

Major Findings: MSF activity could be maintained on EF on Sephadex, in contradistinction to previous findings on polyacrylamide. The pI of MSF was 5, not 7 as had been previously found on flat-bed EF. A value of 7 results from exposure of MSF to temperatures higher than 0-5°C, and is presumably due to an enzymatic transformation.

Significance: The finding of a common pI, 5, rather than an uncommon one, 7, forces us to abandon the previous strategy of isolating MSF by EF. However, the finding of a temperature-dependent transformation provides a new specific isolation strategy.

Proposed Course: Isolation of serum MSF by EF after a room-temperature step (e.g. Sephadex G-200 chromatography) providing quantitative transformation to pI 7 protein.

3) A zwitterionic gel (in collaboration with L. Hjelmeland):

Objectives: To bridge conductance gaps on EF gels which interfere with

the free electrophoretic migration of proteins to their pI-positions, and are therefore a basic problem in protein fractionation by EF.

Methods Employed: Synthesis of a monomer carrying a salt-pair, which could participate in vinyl-polymerization and thus in the formation of cross-linked polyacrylamide gels.

Major Findings: A gel entirely made of monomers carrying a salt-pair exhibits a conductance gap at pH 6 in a pH gradient between 3.5 and 10 which is indistinguishable from that found in polyacrylamide. Thus, the addition of 0.1 M KCl to all phases of an EF system remains the sole remedy to date for conductance gaps, at the price of a considerable increase in EF time.

Significance to Biomedical Research: A major source of artifact in EF remains without palatable remedy.

Proposed Course: Application of Peltier-effect cooled gels, and thermal control of pulsed power, should allow one to electrofocus in 0.1 M KCl at rates comparable to those of low ionic strength EF.

4) Preparative elution-PAGE of hCG, using restacking (in collaboration with G. Kapadia):

Objectives: To prepare hCG of high specific activity from crude commercial starting material in the least laborious fashion.

Method Employed: We exploited the fact that the contaminants of hCG in commercial crude preparations have mobilities higher than hCG. Thus it was possible to find stacking conditions in which they remained stacked, while hCG unstacked quantitatively. However, only when electrophoresis proceeded at pH 5, were proteins other than hCG not unstacked. However, at this pH, hCG separates into 6 major isohormone components, as it does on EF. To simplify the elution of these unstacked isohormones, and avoid undue dilution in elution-PAGE, these 6 components were recombined into a "re-stack" prior to elution, taking advantage for the first time of this possibility which had been theoretically postulated by Jovin.

Major Findings: In a single step, hCG with 8-12,000 IU/mg could be isolated from starting material with 3,000 IU/mg. The yield was 20% on a weight basis.

Significance for Biomedical Research: Researchers in the field of hCG structure-function are being given a simple tool to prepare milligram amounts of homogeneous hCG. A new preparative tool is being introduced. (See also Z01 HD 00165-05 RR).

Proposed Course: Application of restacking to the hGH system: selective stacking of (cationic) hGH-B needed as a bioassay standard, and restacking of the unstacked (cationic) hGH-C, D and E for efficient recovery.

5) Protein extraction and concentration by Steady-State Stacking on an agarose matrix (in collaboration with N. Y. Nguyen):

Objectives: To raise the purity of proteins isolated by extraction and concentration from gel slices, using Steady-State Stacking, from about 50% to 90% or more.

Methods Employed: We took advantage of the recent availability of completely electroendosmosis-free agarose and of the recent realization that agarose can be made to adhere firmly to glass walls once these are coated with a thin film of dried agarose. Thus, the previously described "funnel technique" for electrophoretic extraction of gel slices was carried out on agarose gel cylinders.

Major Findings: Using the agarose procedure, the purity of isolated proteins is increased to 90%.

Significance to Biomedical Research: The new step provides a simple tool to isolate any protein in milligram amounts from a gel by slice excision with a yield of 70% and a purity of the lyophilized product of 90%.

Publications:

Nguyen, N. Y., and Chrambach, A.: Analytical and Preparative Gel Isotachophoresis of Macromolecules. In Hames, B. D., and Rickwood, D. (Eds.): Gel Electrophoresis: A Practical Approach. London, Information Retrieval, Ltd., 1980, in press.

Chrambach, A., and Rodbard, D.: Quantitative and Preparative Polyacrylamide Gel Electrophoresis. In Hames, B. D., and Rickwood, D. (Eds.): Gel Electrophoresis: A Practical Approach. London, Information Retrieval, Ltd., 1980, in press.

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Kapadia, G., Vaitukaitis, J., and Chrambach, A.: See Z01 HD 00165-05 RR.

Chrambach, A.: Electrophoresis and Electrofocusing on Polyacrylamide Gel in the Study of Native Macromolecules. J. Molecular and Cell. Biochem. 29: 23-46, 1980.

Chen, B., and Chrambach, A.: The Effect of SDS on Protein Zone Dispersion in Polyacrylamide Gel Electrophoresis. Anal. Biochem. 102: 409-418, 1980.

Nguyen, N. Y., and Chrambach, A.: Electrofocusing on Flat pH Gradients: Systematic pH Gradient Modification Leading to Improved Protein Separation. Electrophoresis 1: 14-22, 1980.

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Nguyen, N. Y., DiFonzo, J., and Chrambach, A.: Protein Recovery from Gel Slices by Steady-State Stacking: An Apparatus for the Simultaneous Extraction and Concentration of Ten Samples. Anal. Biochem. 106, 78-91, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00173-04 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Gel Electrophoretic Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	N. Y. Nguyen	Visiting Scientist	ERRB, NICHD
Other	A. Chrambach	Senior Investigator	ERRB, NICHD
	R. E. Grindeland	Senior Investigator	BRD, NASA
	G. Baumann	Staff Physician	NUMS, Illinois
	D. Arbegast	Summer Student	

COOPERATING UNITS (if any)

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Northwestern University Medical School, Illinois

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) An optimally flattened pH gradient was generated in electrofocusing through systematic variation of carrier constituent mixtures and selection of anolyte and catholyte. 2) Protein separation was demonstrated to be superior on flat pH gradients, compared to the separation on steep ones. 3) Biologically and immunologically active isohormones D and E of human growth hormone were isolated in milligram amounts by electrofocusing. 4) Replacement of polyacrylamide in Steady-State Stacking by agarose as the anticonvectant was found to improve the purity of final fractionation products. 5) Isolation of biologically and immunologically active isohormones D and E of human growth hormone, in milligram amounts, was achieved by Isotachophoresis.

PHS-6040

1. Gel electrofocusing on flat pH gradients (in collaboration with A. Chrambach): A pH gradient, providing maximal separation between pH 4.7 and 4.9 is generated. The pH gradient is sufficiently flattened to make most of the gel length available for separation. Among the techniques of pH gradient flattening, both a proper selection of carrier constituents and of anolyte proved effective. The nature of the catholyte appears to have no effect on the pH range of the pH gradient.

2. Improved protein separation on flat pH gradients (in collaboration with A. Chrambach): Separation between proteins exhibiting pI values of 4.7 and 4.9 increases in proportion to the increased degree of pH gradient flattening. However, the distribution overlap between closely related protein species may set a limit to the preparative advantage of pH gradient flattening.

3. Milligram-preparative isolation of immunologically and biologically active isohormones D and E of human growth hormone (in collaboration with R. E. Grindeland): A pH gradient, optimally flattened for the isolation of human growth hormone isohormones D and E is generated between pH's 4.3 and 5.6. Separation of the two isohormone species follows a preparative three-step procedure, previously developed, which consists of a) fractionation by electrofocusing on polyacrylamide gel; b) slicing of protein zones; concentration and extraction of protein from gel slices by Steady-State Stacking; c) purification and buffer exchange by gel filtration followed by lyophilization. Total recovery of both isohormone species (15.6 mg) represents 60-70% of the starting protein concentration.

4. Improvement in purity of fractionation products (in collaboration with R. E. Grindeland): Replacement of polyacrylamide gel in Steady-State Stacking by an agarose gel increases the purity of human growth hormone isohormones D and E. This application of agarose gel to Steady-State Stacking has become possible due to the finding that agarose gel can support its weight in glass tubes once the tubes are coated with a thin layer of dried agarose.

5. Milligram-preparative isolation of human growth hormone isohormones D and E by Isotachophoresis (in collaboration with G. Baumann, R. E. Grindeland, D. Arbegast and A. Chrambach): Isotachophoresis excels in load capacity and yield, compared to electrofocusing.

Plan for 1981:

Apply the preparative three-step isolation procedure to a variety of proteins of biological importance such as calliphorin and human growth hormone E.

Isolate homogeneous hGH-B from a complex mixture of several isohormone species by selective stacking or selective unstacking. After partial enzymatic hydrolysis of hGH-B, isolate human growth hormone E in amounts sufficient for physiological studies as well as for bioassay in humans.

Publications:

Nguyen, N. Y., and Chrambach, A.: Electrofocusing on Flat pH Gradients: Systematic pH Modification Leading to Improved Protein Separation. Electrophoresis 1: 14-22, 1980.

Nguyen, N. Y., DiFonzo, J., and Chrambach, A.: Protein Recovery from Gel Slices by Steady-State Stacking: An Apparatus for the Simultaneous Extraction and Concentration of Ten Samples. Anal. Biochem. 106: 78-91, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00174-04 RR

PERIOD COVERED

October 1, 1979 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Hormonal Control of Ovarian Proteoglycan Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Senior Investigator	ERRB, NICHD
Other	M. Yanagishita	Visiting Fellow	ERRB, NICHD
	V. C. Hascall	Senior Investigator	LB, NIDR

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NIDR

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Biophysical Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The proteoglycans present in porcine and rat follicular fluid have been characterized with regard to their chemical and physical-chemical properties. The proteoglycans, approximately 2 million molecular weight, consist of 20% protein, 55% dermatan sulfate and 20% oligosaccharides. The protein core, approximately 400,000 molecular weight, has significantly different amino acid composition and susceptibility to various proteolytic enzymes from those of cartilage-type proteoglycans. The dermatan sulfate chains are approximately 56,000 in molecular weight, and iduronic acid accounts for 9% of total hexuronic acid. The oligosaccharides, approximately 350 chains per molecule, consist of penta and hexasaccharides, and are linked to the protein core by the O-glycosidic bonds. The chemical composition of these oligosaccharides has been determined. The oligosaccharides resemble those found in mucin-type glycoproteins. There is no apparent change in proteoglycan molecules with the size of follicles. Further, we have demonstrated that in vitro cultured granulosa cells produce several species of proteoglycans in addition to those found in porcine follicular fluid.

PHS-6040
(Rev. 10-76)

Objectives:

This study is intended to examine biochemical mechanisms of ovarian responsiveness to follicle stimulating hormone, luteinizing hormone, human chorionic gonadotropin, and pregnant mares serum gonadotropin (PMSG). The molecular structure of ovarian proteoglycans (a unique constituent of the follicular fluid) is to be studied, as well as the effects of hormonal manipulations on the biosynthesis and physico-chemical properties of these proteoglycans.

Methods Employed:

1. Protein isolation, involving cesium chloride density gradient sedimentation under dissociating conditions, gel filtration involving Sepharose and Sephadex, polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE.

2. Chemical characterization of protein and carbohydrate moieties of proteoglycans, using enzymatic, colorimetric, and chromatographic methods.

Major Findings:

Monomer proteoglycan was isolated from porcine ovarian follicular by isopycnic CsCl centrifugation in the presence of 4 M guanidine HCl and protease inhibitors. The elution profile of the D1 preparation on Sepharose 2B was similar to that of monomer proteoglycan from bovine nasal cartilage, indicating a similar molecular size. Follicular fluid proteoglycans consist of about 20% protein, 55% dermatan sulfate and 20% oligosaccharides rich in sialic acid, galactose, mannose, glucosamine and galactosamine. The amino acid composition of this proteoglycan is significantly different from that of cartilage proteoglycans, with a higher proportion of aspartic acid, threonine and lysine and lower amounts of proline and glycine. Alkali released dermatan sulfate chains are larger on Sepharose 6B (average MW = 56,000) than chondroitin sulfate chains from cartilage proteoglycans (average MW = 25,000), and iduronic acid accounts for 9% of total hexuronic acid. Disaccharide units released by chondroitinase ABC consists of 67% 4-sulfated, 22% 6-sulfated, 5% non-sulfated and 5% disulfated disaccharides. After treatment with 0.05 M NaOH, 1 M $NaBH_4$ at 45° for 25 hr, two major sialic acid containing oligosaccharides were observed on Sephadex G-25, corresponding to penta- and hexasaccharides. The pentasaccharide contained sialic acid, galactose, glucosamine and galactosamine in the proportions 1:2:1:1. The galactosamine is O-glycosidically linked to the protein core. This oligosaccharide accounts for approximately 77% of all the sialic acid in the follicular fluid proteoglycans. The hexasaccharide fraction contained sialic acid, galactose, mannose and glucosamine in the proportions 1:2:1:2. It also contained a small amount of fucose and galactosamine. The linkage of these oligosaccharides to the protein core remains to be determined. The follicular fluid proteoglycans, unlike those from cartilage, do not interact with hyaluronic acid. Digestion with trypsin, chymotrypsin or plasmin released dermatan sulfate-peptides nearly as small as those released by papain or alkali; in contrast, cartilage

proteoglycans were resistant to plasmin and released peptides containing an average of more than 4 chondroitin sulfate chains after trypsin or chymotrypsin digestion.

Studies by others (Strickland and Beers) have shown that the ovarian follicular fluid contains a high level of plasminogen activator immediately prior to ovulation. While this enzyme may conceivably be responsible for partial degradation of the follicular wall, it also appears likely that the soluble proteoglycans would also be affected. However, the apparent osmotic gradient which can be generated by complete proteolysis of the protein core, with release of the osmotically active polysaccharide side chains, is limited because of the low concentration of the proteoglycan in the follicular fluid. However, such proteolysis would also be responsible for a decrease in viscosity and, if similar proteoglycans are responsible for the formation of the cumulus oophorus, then such proteolysis may conceivably be responsible, in part, for the release of the ovum.

In related studies, we have demonstrated that in granulosa cell cultures derived from hypophysectomized immature female rats treated with diethylstilbestrol, or in granulosa cell cultures derived from animals previously treated with pregnant mares serum gonadotropin (PMSG), the addition of FSH in vitro results in a significant increase in 35_S sulfate incorporation into macromolecules. Using the same experimental system, we have also demonstrated that granulosa cells produce several different species of proteoglycans in addition to those found in porcine follicular fluid.

Proposed Course:

Further studies will focus on the in vitro model systems, utilizing granulosa cells from the HIFR-DES model and from the PMSG model. The role of proteoglycans in follicular growth, development, atresia and ovulation will be studied. Further, chemical characterization of the oligosaccharides and of the nature of biosynthesis of these materials will be studied.

Publications:

Yanagishita, M., Rodbard, D., and Hascall, V. C.: Isolation and Characterization of Proteoglycans from Porcine Ovarian Follicular Fluid. Journal of Biological Chemistry. 254(3):911-920, 1979.

Rodbard, D.: Agonist versus Antagonist. Trends in Pharmacological Sciences 1 (9), 222-225, 1980

Yanagishita, M., Hascall, V.: Biosyntheses of Proteoglycans by rat granulosa cells cultured in vitro, J. Biol. Chem. 254: 12355, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00175-03 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Gel Electrophoresis with Continuous Optical Scanning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Betty Chen	Senior Staff Fellow	ERRB, NICHD
Other	David Rodbard	Senior Investigator	ERRB, NICHD
	Andreas Chrambach	Senior Investigator	ERRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

By application of the continuous optical scanning to polyacrylamide gel electrophoresis (PAGE), we have been able to 1) estimate the apparent diffusion coefficient (D') of a single protein in gel, and 2) empirically formulate the relationships between D' and a set of physical parameters such as current density, protein load, gel concentrations and ionic strength. The method has been further extended to study 1) the electrophoretic properties of micelles formed by sodium dodecyl sulfate (SDS) and lithium dodecyl sulfate (LDS), 2) the separation and characterization of protein-pigment complexes by SDS-PAGE using a mixture of ragweed pollen antigen as a model compound, and 3) the physical properties associated with agarose gel electrophoresis. Electrophoresis in polyacrylamide was compared with that in agarose gel under identical experimental conditions. Model compounds used for investigation are the peptide, adrenocorticotropic hormone (ACTH), and the protein mixture of ragweed pollen antigens.

Objectives:

To make use of the optical properties of proteins and gel buffer systems in the studies of protein separations aimed at the analysis of membrane protein systems. The method will be developed further in regard to other supporting media for electrophoresis such as agarose gel.

Major Findings:

1. Electrophoretic properties of SDS.

We have reported the improvement in the sharpness of SDS-protein zones in a gel electrophoresis when SDS was present in the upper buffer. Another aspect which plays an equally important role in SDS-PAGE is electrophoretic properties of SDS molecules themselves. A gel (5% T 15% C_{DATD} for stacking gel and 10% T 2% C_{Bis} for the resolving gel) buffered by GABA-BICINE-AMMEDIOL with pH 9.5-11.0, was chosen, since the gel buffer system generated sharp zones for the moving boundary and SDS micelles which can be detected optically. The relative electrophoretic mobility of SDS micelles was found to be dependent on the pH value of the lower gel buffer. At lower pH (less than 10.0), the stack of SDS micelles migrated to the leading edge of the moving boundary, while the reverse order was found at increasing pH of the gel buffer. The relationships between the SDS stack and various SDS concentrations in the upper buffer were investigated.

2. Separation and characterization of protein-pigment complexes by SDS-PAGE.

Separation of a native protein mixture and SDS protein derivatives were carried out in the PAGE apparatus with continuous optical scanning. Ten repetitive optical scans were taken to monitor the region (5 cm along the resolving gel) where a protein stack diffused into separated zones. For the case of native protein mixtures, the dissociated free pigments formed sharp zones and migrated ahead of the moving boundary, while the undissociated pigments migrated randomly behind the moving boundary. In the case of SDS-protein in SDS-PAGE, mixed micelles formed by free pigments and SDS migrated ahead of the moving boundary, and the remaining SDS-proteins were resolved into discrete zones (4-5) migrating behind the moving boundary.

Proposed Course:

1. Polyacrylamide gel electrophoresis in the presence of lithium dodecyl sulfate (LDS-PAGE).

The preliminary studies on the basis of the same gel buffer system and the same gel concentration indicated that the optical scan of LDS micelles was indistinguishable from that of SDS micelles. Since LDS is more soluble than SDS, LDS will be more suitable for electrophoresis conducted at low temperature and for the sample which requires smaller amounts of detergents. The research area to be undertaken will be 1) to determine time-dependent LDS stack and the rate of LDS micellar formation in terms of the absorbance

at 280 nm, and 2) to estimate the optimal LDS concentration to dissociate the pigments from protein-pigment complexes.

2. Preparative SDS-PAGE.

In an analytical scale run with a protein load of 0.35 mg, the antigen mixture of SDS derivatives was resolved into four major zones. As the protein load increased to 1 mg, protein zones were inseparable due to an overloading. A preparative scale run with a 3.5 mg protein in an 18 mm pyrex gel tube and an analytical scale of 0.35 mg load in a 6 mm quartz gel tube were carried out in a conventional PAGE apparatus. From time to time, the small quartz gel tube was removed and subjected to continuous optical scanning. The preliminary result of our preparative SDS-PAGE on the basis of the stained guide strip along the 18 mm gel indicated that the SDS protein mixture was well resolved into separated zones. Further studies on the correlation of the analytical to the preparative scale runs will be made.

3. Agarose and polyacrylamide gel electrophoresis.

1) The mixture of ragweed pollen antigens.

By comparing a gel of 5% T 15% C_{DATD} with 0.5% agarose under identical protein load, current density, gel buffer and duration of electrophoresis, it was shown that the resolution of protein-pigment complexes in the agarose gel was better than in the polyacrylamide gel. There were five zones resolved in agarose gel and four zones in polyacrylamide gel respectively. All zones contained yellow pigments. Experimental work concerning the relationships between pore sizes and agarose gel concentrations will be carried out in the future.

2) Peptide ACTH (N-terminal 24 amino acid residues).

A sharp zone with 280 nm absorbance of 0.2 migrated at the leading edge of the moving boundary was observed in the agarose as well as in the polyacrylamide gel for a peptide load of 20 µg. The physical parameters related to migration distance, zone dispersion, velocity, and peak shape are being evaluated in the present study.

Publications:

Chen, B., Chrambach, A., and Rodbard, D.: Continuous Optical Scanning in Polyacrylamide Gel Electrophoresis: Estimation of the Apparent Diffusion Coefficient of Lactoglobulin B. Anal. Biochem. 97: 120-130, 1979.

Chen, B., and Chrambach, A.: The Effect of SDS on the Protein Zone Dispersion in Polyacrylamide Gel Electrophoresis. Anal. Biochem. 102: 409-418, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00176-03 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Evaluation of Adrenocortical Hyperfunction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI Charles A. Strott Senior Investigator NICHD, ERRB

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A regimen of standard tests for the diagnosis and differential diagnosis of the hyperfunctioning pituitary-adrenal axis has been designed to provide data for comparison of the various determinations and procedures now commonly employed. In addition the results of these tests will be compared to investigative procedures to be carried out under protocols which will be separately submitted. Patients will be treated by standard clinical methods, or by investigative methods also to be covered by future protocols.

PHS-6040
(Rev. 10-76)

Project Description

The clinical features of the hyperfunctioning pituitary-adrenal axis, standard diagnostic tests, and principles of treatment have been elucidated by several centers and serve as the basis for management of most patients with Cushing's syndrome, primary aldosteronism, and the excessive production of androgens or estrogens. However, numerous problems remain. Such disorders are not common, but the symptoms and signs which can be associated with them, e.g., fatigue, weakness, hypertension, obesity, osteoporosis, hirsutism, glucose intolerance, etc., are relatively common; thus, evaluation for adrenal overactivity is likely, in many instances, to be unrewarding. Numerous "screening tests" which have been suggested are either unreliable, or excessively expensive. Although the history and physical examination are frequently adequate when dealing with a well-developed syndrome, the variety and subtlety of the presentations of these disorders are well-recognized. Recent experience on our ward has demonstrated that the usual baseline determinations and suppression tests repeated on the same patient may be variable and difficult to interpret. Periodic hormonogenesis is a feature of many of these disorders; thus, long hospitalizations and numerous determinations are frequently necessary.

Careful attention to the details of the various testing procedures and minor alterations in the performance of these tests have also led to a better understanding of the physiology of the normal hypothalmic-pituitary-adrenal axis and the pathophysiology of steroid secretion in disease states in which the adrenal may be primarily or secondarily involved. In addition, advances in the field of neuroendocrinology have made it desirable to continue to observe the behavior of certain peptides whose secretion is closely associated with that of adrenocorticotrophic hormone.

The present protocol is designed to compare several diagnostic tests commonly used in the evaluation of possible adrenocortical hyperfunction and to provide clinical material for the development of improved tests and procedures. The same patients will become the subjects of further protocols designed to delineate the pathophysiology of the various disease states and to evaluate the various modes of therapy. Until those protocols are operational, patients will be treated by standard clinical guidelines.

Patient selection will be directed primarily toward Cushing's syndrome, but the design of the protocol is general enough to include hypersecretion of any adrenocortical hormone. The basis for admission is the clinical suspicion of hypersecretion; but, when possible, appropriate steroid measurements will have been performed prior to admission.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00179-02 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Inferior Petrosal Sinus Catheterization in Diagnosis and Treatment of Pituitary Tumors.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	NICHD, ERRB
Other	John R. Doppman	Chief, Diagnostic	CC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Adrenal

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inferior Petrosal sinus opacification by retrograde venography has been used at several centers as an alternate technique for opacification of orbital veins. In addition, the procedure delineates the anatomy of the cavernous and intrasellar venous sinuses and provides an opportunity for obtaining blood samples directly from the cavernous sinuses. We propose that it may offer definitive methods for the diagnosis of pituitary tumors and that sampling the effluent for hormones will provide evidence for the source of hypersecreted hormones when that source is in doubt. To establish whether this be the case, patients with disorders of the pituitary-adrenal axis who are undergoing continuous venous catheterization for other reasons will have sampling of the inferior petrosal sinus for hormone measurement, and, when indicated opacification of the cavernous sinus.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00186-01 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Adrenocortical Cells in Tissue Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	ERRB, NICHD
	Kenji Fujieda	Visiting Fellow	ERRB, NICHD
	Alan Goff	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adrenocortical cells are being grown in tissue culture to examine their sterordogenic response to various manipulations. To date these cells have been obtained primarily from the rat, monkey, and guinea pig. Preliminary results have shown that there is a striking difference in sensitivity to ACTH when cells from these three species were compared. An explanation for this difference is being sought. In additon, we are currently comparing rat (dexamethasone-sensitive) and guinea pig (dexamethasone-resistent) responses in vito to similar responses in vivo.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00184-02 RR

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of Pituitary Hormone Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt, Chief	ERRB, NICHD
Other	R. L. Clayton, Visiting Scientist	ERRB, NICHD
	Z. Naor, Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines the mechanisms by which gonadotropin releasing hormone (GnRH) controls the secretion of LH and FSH by anterior pituitary cells. This includes characterization and analysis of the GnRH receptors in anterior pituitary cells and membranes, and the role of calcium, cyclic nucleotides and other intracellular regulators in the mechanism of action of GnRH.

PHS-6040

Objectives:

To characterize the GnRH receptors and elucidate the mechanism by which receptor occupancy leads to release of gonadotropic hormones.

Methods Employed:

Radioligand assay of GnRH receptors in pituitary homogenates and cultured cells. Measurement of gonadotropins and cyclic nucleotides by radioimmunoassay.

Major Findings:

1. Characteristics of cellular GnRH receptors.

The properties of GnRH receptors were analyzed in isolated pituitary cells prepared by enzymatic dispersion with trypsin or collagenase-hyaluronidase. Specific binding sites for GnRH, assayed by equilibration with [^{125}I]iodo[D-Ser (t-but)^{6}]des-Gly^{10}-GnRH N-ethylamide (GnRH-A), were only demonstrable in collagenase-dispersed cells, both initially and after two days in culture. Cellular uptake of GnRH-A was temperature-dependent, with rapid and saturable binding to a limited number of specific receptor sites (about 2000 per gonadotroph) with high affinity for the labeled analog (K_a = 4.0 ± 0.8 x 10^9 M^{-1}). These sites showed common binding specificity for GnRH-A and the native GnRH peptide, with significantly lower affinity for the natural peptide (K_a = 2.3 x 10^7 M^{-1}). Other protein and peptide hormones, including oLH, oPRL, hCG, TRH, somatostatin and angiotensin II (up to 10^{-6}M) did not inhibit binding of GnRH-A to its receptors. Cellular binding of GnRH-A was followed by increased cGMP production and LH release within 10 min. The analog was 50 times more potent than native GnRH in stimulating LH release. The K_{act} values based upon LH release studies were 0.5 and 0.01 nM, respectively, considerably lower than the Kd values of 50 and 0.25 nM derived from receptor binding analysis. These results indicate that GnRH receptors can be identified in isolated pituitary cells, in which peptide binding is followed by increased cGMP production and LH release. The impaired LH responses in acutely-dispersed pituitary cells are not due to loss of receptor sites but to a reversible post-receptor defect. Occupancy of about 20% of the GnRH binding sites elicits a near-maximal LH response, indicating the non-linearity of GnRH-receptor coupling to secretory responses in the cultured gonadotroph.

2. Role of cyclic nucleotides in GnRH action.

The independence of GnRH action from cAMP production was previously demonstrated in cultured gonadotropes. A role of cyclic cGMP in GnRH action has also been suggested by the ability of the releasing hormone to raise cGMP levels in pituitary cells. Gonadotropin releasing hormone (GnRH) and its potent analog [D-Ser(tBu)^{6}] des-Gly^{10}-GnRH N-ethylamide were found to elevate pituitary cyclic GMP levels while stimulating gonadotropin release in cultured pituitary cells. Addition of mycophenolic acid to pituitary cell cultures decreased basal and GnRH induced cGMP production to undetectable levels, but did not reduce basal or GnRH-stimulated luteinizing hormone (LH)

release. Elevation of endogenous cGMP levels by sodium nitroprusside, or addition of cGMP or its potent derivatives, was also without effect on basal or GnRH-stimulated LH release. These findings demonstrate that the elevation of intracellular cGMP during GnRH action does not mediate the release of LH by pituitary cells.

3. Analysis of GnRH analogs by radioligand-receptor assay.

The recently developed radioligand-receptor assay for pituitary gonadotropin releasing hormone (GnRH) receptors, employing a radioiodinated superagonist analog as the ligand, was used to analyze the receptor-binding properties of several GnRH analogs. This receptor assay is specific for GnRH and related peptides with agonist or antagonist activity. All superagonist analogs had equilibrium association constants (Ka) between 4- and 8-fold greater than that of the natural GnRH decapeptide ($Ka = 6.6x10^{8}M^{-1}$). Conversely, weak agonists and inactive analogs were bound with affinities 0.003-0.05 times that of GnRH. Weak antagonists, such as [D-Phe^{2}]GnRH and [D-Phe^{2}, D-Phe^{6}]GnRH, had lower binding constants, while the most potent antagonist, [D-pGlu^{1}, D-Phe^{2}, D-Trp^{3,6}]GnRH, exhibited 8-fold higher affinity than GnRH for pituitary receptors. There was a general positive correlation between receptor-binding affinity and relative biological activity, whether agonist or antagonist, for the several GnRH analogs tested. Comparison of receptor binding data with structural features of the GnRH analogs revealed that the three N-terminal amino acids are critical for receptor recognition, while the C-terminal des-Gly^{10} ethylamide modification does not enhance binding affinity. The radioligand assay provides a rapid and reliable method for evaluating the contribution of receptor-binding affinity to the agonist or antagonist actions of new GnRH analogs. This procedure also permits systematic determination of the structural requirements for GnRH receptor recognition.

Significance:

Clarification of the mechanisms of action of GnRH should improve understanding of pituitary disorders and permit the development of improved methods for the control of gonadotropin secretion.

Proposed Course:

The relationship between GnRH binding and LH release will be further evaluated with agonist and antagonist analogs of the releasing hormone. The role of the GnRH receptors during sexual maturation and the ovarian cycle and pregnancy will be explored in detail, and the effects of agonists and antagonists on these processes will be analyzed. The calcium-dependent mechanism by which GnRH stimulate LH release will be investigated to evaluate the role of phospholipid metabolites including arachidonic acid and its products, in the actions of GnRH upon the gonadotroph.

Publications:

Clayton, R. N., Shakespear, R. A., Duncan, J. A. and Marshall, J. C.: Radioiodinated non-degradable gonadotropin-releasing hormone analogs; new probes for the investigation of pituitary gonadotropin releasing hormone receptors. Endocrinology 105: 1369-1381, 1979.

Clayton, R. N., and Catt, K. J.: Receptor-binding affinity of gonadotropin-releasing hormone analogs: analysis by radioligand-receptor assay. Endocrinology 106: 1154-1159, 1980.

Naor, Zvi, and Catt, Kevin J.: Independent actions of gonadotropin releasing hormone upon cyclic GMP production and luteinizing hormone release. The Journal of Biological Chemistry 255: 342-344, 1980.

Naor, Zvi, Clayton, Richard N., and Catt, Kevin J.: Characterization of GnRH receptors in cultured rat pituitary cells. Endocrinology, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00185-01 RR

PERIOD COVERED

October 1, 1979 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of Temperature and Other Physical Factors on Localization, Natural History and Susceptibility to Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
Other	G. del Negro,	Professor, Department of Medicine	
	S. Imamura	Chief, Department of Physical Medicine	

COOPERATING UNITS (if any)

Hospital das Clinicas, University of Sao Paulo, Sao Paulo Brazil

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Biophysical Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of the role of temperature and of fever on disease in man and experimental animals has been reviewed, using methods of library research. It appears that the localization, natural history, and susceptibility to disease is closely governed by temperature. This applies not only to infectious disease (bacterial, mycobacterial, fungal, protozoal, rickettsial, viral, helminthic), but also to metabolic and immunological diseases. This has suggested the need for a renewed look at the role of fever therapy or local hyperthermia in a variety of disease states.

PHS-6040
(Rev. 10-76)

Objectives:

This project is intended to evaluate the role of temperature, fever, hyperthermia and hypothermia on the course of disease in man and experimental animals. Such information may assist in the physical diagnosis of the disease, in the design of clinical and epidemiological studies, and in the choice of experimental animals.

Methods Employed:

Primarily library research methods and literature review have been employed. However, it is anticipated that clinical studies will be developed in cooperation with the Departments of Infectious Diseases and of Physical Medicine at the Hospital das Clinicas, Sao Paulo Brazil, and with the Laboratory of Parasitic Diseases, NIAID. We also plan laboratory studies, e.g. of the temperature dependence of antigen-antibody reactions.

Major Findings:

The role of temperature in modulating disease has been investigated for the past 100 years. During the period when fever therapy was actively utilizied for syphilis and gonorrhea, the effect of fever was highly regarded. Then, with the development of penicillin and other antibiotics, nonspecific "fever therapy" was generally disregaded, and disparaged. For the past twenty years, most workers have argued that there is relatively little if any role for fever in providing host defense against infectious diseases. However, a review of the literature in an in-depth study, suggests that the localization of lesions is highly governed by temperature. This applies not only to leprosy, to other mycobacterial diseases, to the venereal diseases, but also to several parasitic diseases including leishmaniasis, and a variety of metabolic diseases. Temperature is probably one factor modulating the localization of lesions in gout, pseudo-gout, cystinosis, hyperlipoproteinemia, and several of the immunological diseases, ranging from rheumatoid nodules to certain types of drug reactions.

Significance to Biomedical Research and the Program of the Institute:

The studies here, involving the role of physical factors in the localization of diseases, has considerable impact on many areas of medicine, ranging from infectious disease to immunological diseases, metabolic diseases, etc. It relates directly to the question, as to whether antipyretic therapy is advisable. In particular, several of the diseases which were reviewed here, including leprosy and certain rickettsial diseases and fungal diseases result in chronic indolent infections of the testes (chronic orchitis). This is often accompanied by endocrine disorders including gynecomastia. One of the reasons the testes are susceptible to these diseases, may be the fact that the temperature of the testes is several degrees cooler than core body temperature. Further, the temperature of the testes and scrotum are very well regulated at approximately 32 to 34 degrees. This provides an optimal environment for those organisms which grow best at this temperature (e.g. Mycobaterium leprae,

Paracoccidioides brasiliensis). The lesions of paracoccidiomycosis are generally distributed over cool regions. This disease also has a relatively high incidence of involvement of the adrenal glands, thus contributing to the frequent occurrence of Addison' disease.

Proposed Course:

Discussions are now under way with members of the faculity of Hospital das Clinicas in the University of Sao Paulo, to develop treatment programs for leishmaniasis, paracoccidiomycosis, and possibly leprosy. Additional discussions are underway with members of NIAID. By measurements local body temperature, employing thermography, and cutaneous thermomisters, we shall examine the temperature at sites of active lesions, in a variety of disease states. The thermographic equipment will also be used to evaluate patients with Leydig-cell tumors and infertility associated with varicocoele.

Publications:

Rodbard, D., Wachslicht-Rodbard, H., and Rodbard, S.: Temperature: A critical factor determining localization and natural history of infectious, metabolic and immunological diseases. Perspectives in Biology and Medicine 23: 439-474, 1980.

Rodbard, D.: Treatment of Mucocutaneous Leishmanisais (Letter to the Editor). The New England Journal of Medicine 300 (26): 1489, 1979.

Del Negro, G., Melo, E. H. L., Rodbard, D. Melo, M. R., Layton, J., and Wachslicht-Rodbard, H.: Limited Adrenal Reserve in Patients with Paracoccidioidomycosis: Cortisol and Aldosterone Responses to Beta-1-24 ACTH. Clinical Endocrinology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00187-01 RR (formerly 00086-10 LBS)

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Hormonal Regulation of Cellular Metabolism by Phosphorylation and Dephosphorylation of Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K.-P. Huang	Research Chemist	ERRB, NICHD
OTHER:	Z. Ahmad	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)
J. Chou, PRB, NICHD; P.B. Chock, LB, NHLBI.

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Topics of current interest are: (1) the regulation of glycogen metabolism in mammalian tissues and cells grown in culture; (2) hormonal regulation of glycogen synthesis in normal and diabetic animals; (3) elucidation of the regulations of phosphorylation and dephosphorylation of glycogen synthase by protein kinases and phosphoprotein phosphatases.

Project Description:

Objectives: The main objectives of this project are: (1) to study the regulatory mechanisms involved in the modification of proteins through phosphorylation and dephosphorylation reactions and (2) to elucidate the hormonal effect on the state of protein phosphorylation in normal and diabetic animals.

Methods Employed: The principal methods employed are: (1) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, ion-exchange chromatography, and affinity chromatography; (2) enzyme assays, which are usually based on spectrophotometric or radiometric methods; (3) characterizations of peptides by gel electrophoresis, isoelectric focusing, and peptide mapping; and (4) tissue culture techniques, including maintenance and manipulation of cells.

Major Findings: (1) Glycogen synthase from rabbit skeletal muscle is phosphorylated by cyclic AMP-independent synthase kinase-1 up to 4 mol phosphate/subunit. The phosphorylation of these four sites proceeds in a sequential order in which a site has to be phosphorylated before the other three sites are phosphorylated. This unique controlling mechanism provides more subtlety to the regulation of glycogen synthase activity. (2) The functional roles of the multiple phophorylation sites of glycogen synthase phosphorylated by cyclic AMP-independent synthase kinase-1 have been defined by comparing the kinetics of the phosphorylation of these sites with the changes in the functions of the enzyme. It was found that a site primarily controls the binding of substrate, UDP-glucose, and three sites control the binding of the allosteric effector, glucose-6-P, to the synthase. (3) The phosphorylation of glycogen synthase by cyclic AMP-dependent protein kinase results in the incorporation of 1.2-1.5 mol phosphate/subunit. Analysis of the ^{32}P-labeled tryptic peptides reveals the presence of two phosphorylation sites. These two sites affect the binding of UDP-glucose to the synthase; however, they have negligible effect on the binding of glucose-6-P to the synthase. (4) Human placental and calf intestinal alkaline phosphatases were found to have phosphoprotein phosphatase activity which preferentially dephosphorylates one site and three sites from synthase phosphorylated by cyclic AMP-dependent protein kinase and cyclic AMP-independent synthase kinase-1, respectively. The specificity inherent in these alkaline phosphatases are useful for the study of the functional roles of the phosphorylation sites in phosphoproteins.

Significance to Biomedical Research and the Program of the Institute: Investigation on the regulation of the activity of glycogen synthase, a rate-limiting enzyme for the synthesis of glycogen, provides the understanding of the glycogen metabolism in normal and pathological development. Using glycogen synthase as a model system, which is under control by multiple-sites phosphorylation with multiple kinases, we learn more about the regulation of phosphorylation and dephosphorylation reactions -- key processes in the regulation of gene expression, cellular metabolism, differentiation, and growth. The identification of the functional roles of the various phosphorylation sites in glycogen synthase will be important in determining the specific functional change in glycogen synthase associated with fetal development and diabetes.

Proposed Course of Project: We will continue to explore the regulatory mechanism of phosphorylation and dephosphorylation of glycogen synthase and to apply the findings in the study of the regulation of cellular metabolism associated with fetal development and with diabetes.

Publications:

1. Huang, K.-P.: Regulation of glycogen synthase activity in choriocarcinoma cells. Effect of glucose starvation on the conversion of synthase D to synthase I and the kinetic properties of the enzyme. European. J. Biochem., 95, 477-485, 1979.

2. Itarte, E., and Huang, K.-P.: Purification and properties of cyclic AMP-independent glycogen synthase kinase 1 from rabbit skeletal muscle. J. Biol. Chem., 254, 4052-4057, 1979.

3. Huang, K.-P., Lee, S.-L., and Huang, F.L.: Phosphorylation of rabbit skeletal muscle glycogen synthase I by a cyclic AMP-independent synthase kinase. J. Biol. Chem., 254, 9867-9870, 1979.

4. Huang, K.-P., Huang, F.L.: Dephosphorylation of rabbit skeletal muscle glycogen synthase by phosphoprotein phosphatase and human placental alkaline phosphatase. Biochem. Biophys. Res. Commun., 92, 682-687, 1980.

5. Huang, K.-P., Huang, F.L.: Phosphorylation of rabbit skeletal muscle glycogen synthase by cyclic AMP-dependent protein kinase and dephosphorylation of the synthase by phosphatases. J. Biol. Chem., 255, 3141-3147, 1980.

6. Ahmad, Z., and Huang, K.-P.: Dephosphorylation of rabbit skeletal muscle glycogen synthase (phosphorylated by cyclic AMP-independent synthase kinase-1) by phosphatases. J. Biol. Chem., in press.

1980 ANNUAL REPORT
Endocrinology and Reproduction Research Branch

Project Numbers	Project Title	Principal Investigator
Z01 HD 00021-04	Bioassayable and Radioimmunoassayable ACTH	C. Strott
Z01 HD 00022-07	Renin-Angiotensin System and Aldosterone Regulation	K. Catt
Z01 HD 00035-08	The Structure and Function of Biologically Active Molecules	E. Gross
Z01 HD 00040-05	Experimental and Theoretical Studies of Hormone Receptor Interaction and Response Coupling	D. Rodbard
Z01 HD 00041-05	Cardiovascular Effects of Thyroid, Hormones, Catecholamines and Cardiotoxic Drugs	D. Rodbard
Z01 HD 00042-05	Mathematical and Statistical Theory of Gel Electrophoresis and Filtration	D. Rodbard
Z01 HD 00043-05	Mathematics and Statistics of Radioligand Assays	D. Rodbard
Z01 HD 00146-05	Structure and Function of Chorionic Gonadotropins	H. Chen
Z01 HD 00147-05	Mechanism of Action of Gonadotropins and of ACTH	M. Dufau
Z01 HD 00148-05	Ontogeny of Gonadotropin Receptors and Gonadal Function	K. Catt
Z01 HD 00149-05	Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin	M. Dufau
Z01 HD 00150-05	Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase	M. Dufau
Z01 HD 00151-05	Regulation of Gonadotropin Receptors in Testis and Ovary	K. Catt
Z01 HD 00152-05	Characterization and Radioligand Assay of Gonadotropin Receptors	K. Catt
Z01 HD 00160-05	Biology of the Adrenocortical Cell: Molecular Events in Steroid Biosynthesis and Secretion	C. Strott
Z01 HD 00164-04	Strategy and Mechanisms of Fractionation	N. Nguyen
Z01 HD 00165-05	Isolation and Characterization of Protein Hormones & Other Active Proteins	A. Chrambach
Z01 HD 00170-04	A Randomized Study for the Treatment of Chromophobe Adenomas of the Pituitary Gland with Irradiation	C. Strott
Z01 HD 00171-04	Other PAGE Instrumentation and Procedures	A. Chrambach
Z01 HD 00173-04	Gel Electrofocusing Methods	N. Nguyen
Z01 HD 00174-04	Hormonal Control of Ovarian Proteoglycan Synthesis	D. Rodbard
Z01 HD 00175-03	Gel Elecrophoresis with Continuous Optical Scanning	B. Chen
Z01 HD 00176-03	Clinical Evaluation of Adrenocortical Hyper-Function	C. Strott
Z01 HD 00179-02	Inferior Petrosal Sinus Catherization in Diagnosis and Treatment of Pituitary Tumors	C. Strott

Project Numbers	Project Title	Principal Investigator
Z01 HD 00184-02	Regulation of Pituitary Hormone Secretion.....	K. Catt
Z01 HD 00185-01	Effect of Temperature and Other Physical Factors on Localization, Natural History Susceptibility to Disease.....	D. Rodbard
Z01 HD 00186-01	Studies of Adrenocortical Cells in Tissue Culture..	C. Strott
Z01 HD 00187-01 (Previously Z01 HD 00086-10)	Hormonal Regulation of Cellular Metabolism by Phosphorylation and Dephosphorylation of Proteins..	K.-P. Huang

DEVELOPMENTAL ENDOCRINOLOGY BRANCH

ANNUAL REPORT

October 1, 1979 - September 30, 1980

Project #	Project Title	Principle Investigator
Z01 HD 00157-05	Physiology of Puberty	G. Cutler, Jr., M.D.
Z01 HD 00158-05	Pathophysiology of Gynecomastia and Hirsutism	D.L. Loriaux, M.D.
Z01 HD 00161-05	Role of Sex Steroids in Regulation of FSH and LH Levels in Man	R.J. Sherins, M.D.
Z01 HD 00163-05	Clinical Studies of Male Reproductive Disorders	R.J. Sherins, M.D.
Z01 HD 00180-02	Biology of Hormone Binding Proteins	B.C. Nisula, M.D.
Z01 HD 00181-02	Steroid Antagonists	G. Cutler, Jr., M.D.
Z01 HD 00182-02	Structure, Function, and Physiology of Glycoprotein Hormones	B.C. Nisula, M.D.
Z01 HD 00183-02	Catechol Estrogens: Physiological Effects	D.L. Loriaux, M.D.

ANNUAL REPORT
Summary

DEVELOPMENTAL ENDOCRINOLOGY BRANCH
National Institute of Child Health and Human Development

The research aim of the Developmental Enocrinology Branch is to further understanding of the role of the endocrine system in the complex processes of growth and development. The periods of primary interest include the fetal and neonatal period, puberty, and senility. The current focus of research is the pubertal period. The systems under study are two; the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal axis.

Studies on the hypothalamic-pituitary-gonadal axis are directed toward understanding the process underlying the initiation of LH & FSH secretion that heralds the onset of puberty, the mechanism of action of these glycoprotein hormones, the gonadal response to these hormones, and the roles of the gonadal sex steroids in gametogenesis, central nervous system maturation, breast physiology, hair growth, and skeletal maturation.

Studies directed toward understanding the initiation of LH & FSH secretion have centered, in the past year, about the careful examination of the LH-FSH secretion dynamics of children with true precocious puberty and the comparison of these findings with normal pubertal chlidren. There appears to be clear differences in gonadotropin secretory patterns, particularly in boys with the disorder, that suggest that these subjects do not initiate gonadotropin secretion in the same way as normal children. The secretory patterns in boys, when first seen, are reminiscent of the pattern of adult men. This is not the case with girls. A useful sidelight of this study is the application of a long acting LRF analogue for the treatment of these children. Eight patients have begun treatment with a long-acting LRF analog, to our knowledge the first use of this agent to treat precocious puberty. The results thus far have been promising, with decreased pituitary gonadotropin secretion, decreased sex hormone levels, and favorable clinical changes. Since no generally satisfactory treatment is available for this disorder, these early results hold the promise that this may represent a major therapeutic advance. Future systematic investigation of the dose, frequency, duration, and chemical nature of the anlog used will be necessary to realize the full potential benefit from this new form of therpy.

Studies directed toward understanding the mechanism of action of the glycoprotein hormones have centered about developing more sensitive and reliable assays for these hormones, and examining structure-function relationships between the various glycoprotein hormones.

A highly sensitive and reliable radioimmunoassay for the measurement of hTSH in human serum has been developed. The value of earlier radio-immunoassays for hTSH has been limited principally because of poor sensitivity and specificity. This has been severe enough to preclude reliable measurement of serum hTSH levels in normal people. Our new methodology permits delineation of the entire range of values in normal subjects (0.5 - 4.5 U/ml). Results with this assay show that patients with thyrotoxicosis of diverse etiologies (diffuse toxic goiter, toxic multinodular goiter, subacute thyroiditis, and thyrotoxicosis

factitia) have serum hTSH levels suppressed well below the normal range. The procedure involves the concentration of hTSH from serum by adsorption to concanavalin-A covalently linked to 4B Sepharose, lactoperoxidase iodination of the hTSH, and disequilibrium assay conditions. This has proved to be a valuable diagnostic tool in hyperthyroidism.

Progress has also been made in the study of structure-function relationships among the glycoprotein hormones. Our earlier work in rodents suggested that the selectivity of the interaction between hormone and target cell was relative, not complete. Thus, interstitial cell stimulating hormones like hCG and hLH exhibit a weak intrinsic thyrotropic activity in rodent bioassays. To determine if this concept could be extended to the human species, studies of the interaction of hCG and hLH with human thyroid membranes were carried out. Indeed, both hCG and hLH bind to and stimulate the adenylate cyclase activity of human thyroid membranes. An unexpected result was the finding that hLH is about 65-fold more potent than hCG. As these 2 molecules are otherwise functionally quite similar, it is appealing to attribute this functional difference to the most obvious structural difference, the extra 30 amino acids at the carboxy-terminus of hCG-beta. These observations provide a molecular basis for the thyrotropic activity of hCG and hLH, give rise to a hypothetical role for the extra carboxy-terminal region on hCG, and point to key structure differences between thyroid and testis glycoprotein receptors.

Studies directed toward better understanding the gonadal response to the glycoprotein hormones are several. First, much progress has been made in our ability to measure the biologically important fractions of the circulating sex steroid hormones. Testosterone transport is characterized using a solid phase method. Adsorption of testosterone binding globulin, TeBG, from serum samples onto a solid phase matrix of Con A-Sepharose permits examination of testosterone binding to TeBG both before and after the removal of the endogenous serum albumin. Appropriate Scatchard analysis of this binding allows calculation not only of the TeBG binding capacity, but also the TeBG association constant for binding testosterone and a parameter of albumin binding equivalent to the product of its affinity and capacity for binding testosterone. By applying the law of mass action, these testosterone binding parameters together with the total testosterone concentration can be used to calculate the distribution of circulating testosterone into TeBG bound, albumin bound and unbound concentrations. This methodology has been applied to the study of testosterone transport in normal men and women. These studies demonstrate that women have a significantly higher TeBG binding capacity and a lower association constant than men. There was no difference in the albumin binding parameter between men and women.

Studies on spermatogenesis have centered about "experiments of nature", men exhibiting abnormalities of gametogenesis. Studies in the past year have revealed that the sperm from men with the non-motile sperm syndrome (necrospermia) fail to metabolize fructose, the normal primary energy source for sperm, and have increased specific activity of an endogenous lipid peroxidase, an enzyme capable of producing peroxides of sperm phospholipid. These peroxides promptly and irreversibly immobilize spermatozoa. Human sperm have also recently been shown to contain protein carboxyl-methylase, an enzyme required for chemotaxis by mammalian monocytes and bacteria. This enzyme acts upon a

specific protein methyl acceptor and inactivation of the enzyme leads to failure of characteristic unidirectional motile progression. A deficiency of protein carboxyl methylase activity has been identified in some men with immotile sperm, an observation that suggests this may be a biochemical marker for certain of the male infertility states in which sperm motility is altered.

Studies on the central nervous system effects of the gonadal steroids have centered about the feedback actions of androgens and estrogens on the pituitary secretion of FSH & LH. In particular, we have been interested in the role of sex steroids in the regulation of FSH secretion. We have found that testosterone, given at a dose which maintains mid normal plasma testosterone levels and normal prostate size in the castrate rat, results in normal levels of plasma FSH and LH. This indicates that testicular factors other than sex steroids are not necessary for maintaining FSH and LH levels within the normal range in this species.

When estradiol is given at a dose which increases plasma estradiol levels slightly above normal, plasma LH concentrations are suppressed while FSH levels remain unexpectedly elevated into the castrate range. The data indicate that androgen, not estrogen, is critical in maintaining tonic suppression of FSH secretion.

These data add considerable insight into the mechanism of control of gonadotropin secretion in the male. The results indicate that testosterone can maintain both gonadotropins within the normal range in the absence of other testicular factors and, further, that a selective increase in plasma FSH can be induced when there is subnormal replacement of testosterone but increased replacement of estradiol. The data provide an alternate explation for the inhibin hypothesis.

Another class of steroids that may be important in the regulation of FSH and LH secretion are the catechol estrogens. These are metabolic products of estrone and estradiol with a hydroxyl group at the 2 or 4 position. These steroids have the interesting property of being able to interact with both the catecholamine receptors and estrogen cytosol receptors. It has been postulated that these compounds may be the intermediaries of estrogen, and perhaps even androgen, action in the brain. Little is known, however, about the origin, disposition, plasma concentrations, and biologic activity of these hormones.

Both 2-OH estrone and 2-OH estradiol have been shown to interact with the rat uterine cytosol receptor with about 5% the affinity of estradiol. 2-OH estrone has been shown to be an estrogen <u>agonist</u> in man. Intravenous infusions of between 2 and 6 milligrams daily for 4 days are needed to demonstrate the suppressive effect on gonadotropin secretion. 2-OH estradiol is also an estrogen agonist in man. The 2-OH estradiol effect is demonstrable at doses of 400 - 800 µg a day for 4 days. In the rat, no estrogenic or antiestrogenic activity of 2-OH estrone could be found even at doses more than 500 times the dose that would be predicted to be effective based on receptor affinity studies.

These potency studies revealed a major discrepancy between receptor affinity and minimal effective dose for biologic action. The most likely explanation for this discrepancy is pharmacokinetic. We initiated our studies of the pharmacokinetics of the catechol estrogens by examining the metabolic clearance rates of 2-OH-estrone and 2-OH estradiol in man and rat.

The MCR of 2-OH estrone in man ranges between 20,000 & 40,000 L/day. That of 2-OH estradiol is about half this. These clearance rates are 10-20 times greater than the clearance of any other known naturally occurring steroid. Because liver blood flow is rarely greater than 2000 L/day, and cardiac output is on the order of 7,000 L/day, these data suggest that a large portion of the clearance must be accomplished by the blood itself. Subsequent studies have confirmed that 2-OH estrone and 2-OH estradiol are rapidly metabolized by erythrocyte catechol-O-methyl transferase, and that the reaction rate can account for the rapid clearance of the catechol estrogens from blood.

These findings explain why such large doses of the catechol estrogens are needed to elicit biological effects. Because of the need for large doses of catechol estrogens small amounts of an active contaminant could potentially account for all or part of the observed effects. This probably is the explanation for the controversial actions attributed to the catechol estrogens in the literature. Our findings of estrogen agonism are compatible with the measured MCR's, estrogen receptor affinities, and cannot be attributed to contaminants in our purified reagents.

Previous work with this class of steroids using newly developed radioimmunoassays suggested that plasma 2-OH estrone was present in human blood in concentrations ranging between 20-50 pg/ml in men and up to several hundred pg/ml in pregnant women.

These data for non-pregnant adults are not compatible with the enormous MCR's for these compounds that we have found. Hence, we have developed a modified 2-OH estrogen radioimmunoassay in order to reevaluate the plasma concentration of these steroids. We find that plasma 2-OH estrone & 2-OH estradiol do not circulate in measurable quantities in non-pregnant adults (<10 pg/ml) and range up to 200 pg/ml in pregnant women. This is a considerable revision downward of the previously accepted plasma concentrations for these steroids, but are the only values compatible with the known metabolic clearance of these steroids and the production rates of their known precursors.

Studies on the role of the gonadal sex steroids in the pathophysiology of gynecomastia & hirsutism have yielded some interesting findings.

Two etiologic subgroups of patients with gynecomastia still have an unexplained pathogenesis; pubertal and drug induced gynecomastia. Drug induced gynecomastia can have several theoretical causes: 1 - the drug acts as an estrogen; 2 - the drug acts as an antiandrogen; 3 the drug causes an increased production of estrogen from the testes; 4 - the drug causes a decreased secretion of androgen from the testis.

Over the past several years, examples of drugs acting as estrogens

and anti-androgens have been documented, (i.e., digitoxin, and spironolactone). In the last year an example of each of the other two pathogenetic mechanisms has been tentatively identified. Mysoline, a phenobarbital like anticonvulsant, has been shown to increase the metabolic clearance rate of testosterone. Plasma testosterone levels are then kept in the normal range by an increase in LH secretion. The increased LH secretion is also associated with increased plasma estradiol concentrations, presumably because of increased testicular aromatase activity with consequent increased testicular estradiol secretion.

The second mechanism, decreased testicular testosterone secretion, may be a consequence of o,p'-DDD therapy for Cushing's Disease or adrenal carcinoma. These patients have been found to have low plasma free testosterone levels, increased plasma LH levels and, as a result, increased plasma estradiol concentrations. Studies are underway to document testosterone and estradiol production rates in these patients.

The mechanism outlined for mysoline and o,p'-DDD has important clinical implications in that the gynecomastia could theoretically be prevented with testosterone treatment. Such therapy would be expected to reduce LH secretion and, hence, interstitial aromatization with a resultant decrease in estradiol secretion.

A long standing hypothesis advanced to explain the occurrance of hirsutism in women with normal plasma testosterone concentrations is that they have an increased end organ sensitivity to androgen. This hypothesis has been examined by measuring androgen receptor concentrations in fibroblast cultures from a group of hirsute women with plasma testosterone levels in the normal range. No differences were found between these women and a non-hirsute control group. These studies suggest, but do not prove, that increased sensitivity to androgen at the level of the target organe does not contribute to hirsutism in most patients having this complaint in association with normal plasma testosterone values.

The role of the Hypothalamic-pituitary-adrenal axis in growth and development is also under active study. Our first efforts in this area have been to develop tools and animal models that make controlled studies feasible.

We have spent considerable effort in identifying a glucocorticoid antagonist that is active in vivo. 11-deoxycortisol (S) is a known glucocorticoid antagonist in vitro. It has not previously been shown to be an antagonist in vivo. We found that the reason for the apparent failure of S to act as a glucocorticoid antagonist in vivo is that it undergoes 11-hydroxylation by the adrenal gland, thus being converted to an agonist. This has been confirmed by showing that S retains full antagonist activity in adrenalectomized rats. Work has progressed toward developing effective in vivo antagonists by developing S analogues that resist 11-hydroxylation such as $\Delta^{1},9(11)$S. This compound acts as a glucocorticoid antagonist both in vivo and in vitro. Δ',9(11)s, however, is not sufficiently resistant to 11-hydroxylation to have a potential clinical use. A new analog of 11-deoxycortisol is being synthesized which promises to be completely resistant to 11-hydroxylation. As soon as available this analog will be tested for antiglucocorticoid activity.

Also of interest is our recent identification of an animal species that appears to be almost completely resistant to the biological activity of glucocorticoids. This is the squirrel monkey, a new world primate. The mechanism for this resistance seems to reside in the virtual absence of cytosol glucocorticoid receptor. Alternatively, there may be an abnormal receptor having a very low binding affinity for glucocorticoids, a characteristic which would preclude its measurement in most binding assays. Studies in progress are designed to differentiate between these two possibilities. This model should be very useful in providing clues to the role of glucocorticoids in development.

A number of ongoing studies are directed toward understanding the role of adrenal androgen secretion in growth and development and toward understanding the physiological principles underlying this important feature of adrenal function. Over the past year, studies of adrenal androgen secretion in response to ACTH in adults with pituitary disease have revealed that these patients have a preadrenarchal pattern of adrenal androgen secretion, suggesting that pituitary hormones other than ACTH may be required to maintain the postadrenarchal response. Investigations are underway in the chimpanzee, the only known model for human adrenarche, to determine (1) whether human pituitary extract can initiate adrenarche and (2) whether non-ACTH pituitary factors are required to maintain adrenarche in adult chimpanzees. Ten chimpanzees have been entered into the study. Basal measurements of plasma adrenal androgen levels and cortisol and DHA metabolic clearance rates, have been completed. Follow-up determinations during the experimental period are in progress.

Measurement of 17-hydroxylase, 21-hydroxylase, 3β-hydroxysteroid dehydrogenase/ Δ^5,Δ^4-isomerase, and 17,20-desmolase activities in human adrenal microsomes has revealed that the predominant enzymatic alteration during adrenarche is a marked increase in the activities of both 17-hydroxylase and 17,20-desmolase. This observation focuses attention on the factors that regulate these enzymes and suggests that these changes may offer an experimental endpoint for exploring the mechanism of adrenarche. At present very little is known about how the activity of these enzymes is regulated.

A number of recent studies have suggested that prolactin may be important in regulating adrenal androgen secretion. To investigate the role of prolactin in this process, dehydroepiandrosterone sulfate blood production rates have been measured before and after hypophysectomy and before and during bromocryptine treatment in hyperprolactinemic patients. Each of three initial patients who have completed the study have shown a fall in dehydroepiandrosterone sulfate production rate after prolactin levels were lowered. Additional subjects are now under study to corroborate and extend this interesting finding.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00157-05 DEB (previously RRB)

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Physiology of Puberty

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	G. Cutler, M.D., Senior Investigator	DEB, NICHD, NIH
Other	D. Lynn Loriaux, M.D., Ph.D., Chief	DEB, NICHD, NIH
	R. Schiebinger, M.D., Clinical Associate	DEB, NICHD, NIH
	B. Albertson, HEW Post Doctoral Fellow	DEB, NICHD, NIH
	F. Cassorla, Clinical Associate	DEB, NICHD, NIH
	F. Comite, Clinical Associate	DEB, NICHD, NIH

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	6	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to gain insight into the physiologic processes underlying normal and abnormal pubertal progression. Principal areas of investigation include the mechanisms of adrenarche, the temporal changes in hypothalamic function with respect to gonadotropin regulation, the mechanism of the pubertal growth spurt, and the treatment of idiopathic true precocious puberty with an analog of luteinizing hormone releasing hormone.

Z01 HD 00157-05 DEB
(previously RRB)

Objectives:

This project was started in 1976 in an attempt to begin to understand some of the fundamental processes of pubertal development. The three immediate goals are the study of adrenarche, hypothalamic-pituitary maturation, and the mechanism of the pubertal growth increase. In adition, a newly available analog of luteinizing hormone releasing hormone is being used to treat children with true precocious puberty.

Methods Employed:

1) Adrenarche - Methods consist of steroid radioimmunoassay, determination of steroid metabolic clearance rate and production rate, and enzymatic assay of 17-hydroxylase, 21-hydroxylase, 17,20-desmolase, and 3β-hydroxysteroid dehydrogenase/Δ^5, Δ^4-isomerase in adrenal microsomes.

2) Treatment of true precocious puberty-steroid and peptide hormone radioimmunoassay.

3) Mechanism of the pubertal growth spurt - This project will depend on the accurate measurement of growth over very short time intervals (3 weeks) using the recently invented ulnar measuring device of Dr. Ignatius M. Valk (Nijmegen, Netherlands) which is able to measure the length of the ulna with a standard deviation of $\pm$ 0.2 mm.

Progress:

1) Adrenarche - Studies of adrenal androgen secretion in response to ACTH in adults with pituitary disease have shown a preadrenarchal pattern, suggesting that pituitary hormones other than ACTH may be required to maintain the postadrenarchal response.

Measurement of 17-hydroxylase, 21-hydroxylase, 3β-hydroxysteroid dehydrogenase/Δ^5, Δ^4-isomerase, and 17,20-desmolase activities in human adrenal microsomes has revealed that the predominant enzymatic alteration during adrenarche is a marked increase in the activities of both 17-hydroxylase and 17,20-desmolase.

To investigate the role of prolactin in adrenal androgen secretion, dehydroepiandrosterone (DHA) sulfate blood production rates have been measured before and after hypophysectomy and before and during bromocryptine treatment in hyperprolactinemic patients. Each of three initial patients who have completed the study have shown a fall in DHA sulfate production rate after prolactin levels were lowered.

Investigations are underway in the chimpanzee, the only known model for human adrenarche, to determine (1) whether human pituitary extract can initiate adrenarche and (2) whether non-ACTH pituitary factors are required to maintain adrenarche in adult chimpanzees. Ten chimpanzees have been entered into the study. Basal measurements of plasma adrenal androgen levels and cortisol and DHA metabolic clearance rates, have been

completed. Follow-up determinations during the experimental period are in progress.

2) Treatment of true precocious puberty - Eight patients have begun treatment with a long-acting LHRH analog, to our knowledge the first use of this agent to treat precocious puberty. The results thus far have been promising, with decreased pituitary gonadotropin secretion, decreased sex hormone levels, and favorable clinical changes.

Significance:

1) Adrenarche - adrenal androgens play an important pathogenetic role in certain hormone-dependent cancers such as carcinoma of the breast and prostate, and in a variety of endocrine disorders such as congenital adrenal hyperplasia, premature adrenarche, hirsuitism, and Cushing's syndrome. Increased understanding of the mechanism of adrenarche may lead to new methods of controlling adrenal androgen secretion in these disorders.

The observation that increased 17-hydroxylase and 17,20-desmolase are the major enzymatic changes during adrenarche focuses attention on the factors that regulate these enzymes as a new approach to exploring the mechanism of adrenarche. At present very little is known about how the activity of these enzymes is regulated.

2) Treatment of true precocious puberty - Since no generally satisfactory treatment is available for this disorder, the promising early results of LHRH analog therapy may represent a major therapeutic advance. Future systematic investigation of the dose, frequency, duration, and chemical nature of the analog used will be necessary to realize the full potential benefit from this new form of therapy.

Proposed Course:

1) The investigation of the role of the hypothalamic - pituitary system in the induction and maintenance of adrenarche in the chimpanzee. This study, currently underway in collaboration with the primate research facility at Holloman air Force Base, Alamagordo, New Mexico, will be continued.

2) The investigation of the role of prolactin in adrenal androgen secretion. Additional patients will be added to this study and the investigation will be extended to assess the effect of altering prolactin level on DHA sulfate production rate in normal volunteers.

3) The investigation of the functional properties of the human adrenal zona fasciculata and zona reticularis. Portions of human adrenal zona reticularis and zona fasciculata will be removed from surgical specimens with the aid of a dissecting microscope. Androgen secretion rates will be measured during organ culture *in vitro* to compare directly the ability of each zone to secrete adrenal androgens.

4) The treatment of precocious puberty with a long-acting LHRH analog. Patients currently under treatment will continue to be followed to assess the long-term effects of therapy. New patients will be added to the study so that the effects of differing dose, frequency, and duration of treatment can be evaluated.

5) The investigation of the mechanism for the pubertal growth spurt. Patients with delayed puberty will receive short-term treatment (one week) with estrogen, testosterone, or a non-aromatizable androgen (dihydrotestosterone). The growth response to each steroid will be quantitated using a uniquely sensitive device for the measurement of ulnar growth. Dose-response curves will be determined for each sex steroid to determine which contributes most to the pubertal growth spurt.

Publications:

1. Cutler, G.B., Jr., S.E. Davis, R.E. Johnsonbaugh, and D.L. Loriaux: Dissociation of Cortisol and Adrenal Androgen Secretion in Patients with Secondary Adrenal Insufficiency. J. Clin. Endocrinol. Metab. 49: 604-609, 1979.

2. Lucky, A.W., S.P. Marynick, R.W. Rebar, G.B. Cutler, Jr., M. Glenn, R.E. Johnsonbaugh, and D.L. Loriaux: Replacement Oral Ethinyloestradiol Therapy for Gonadal Dysgenesis: Growth and Adrenal Androgen Studies. Acta Endocrinologica 91: 519-528, 1979.

3. Cutler, G.B., Jr., and D.L. Loriaux: Adrenarche and Its Relationship to the Onset of Puberty. Federation Proceedings 39: 2384-2390, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00158-05 DEB (previously RRB)

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Pathophysiology of Gynecomastia and Hirsutism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Lynn Loriaux, M.D., Ph.D., Chief,	DEB, NICHD, NIH
Other	G. Cutler, Jr., M.D., Medical Officer	DEB, NICHD, NIH
	C. Eil, M.D., Clinical Associate	DEB, NICHD, NIH
	G. Merriam, M.D., Clinical Associate	DEB, NICHD, NIH
	J. Booth, M.D., Quest Worker	DEB, NICHD, NIH

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

Developmental Endocrinology Branch

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) XX HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide information for the understanding of disordered phenotypic secondary sexual expression, including such disorders as gynecomastia and hirsutism. The current principal area of investigation is end organ steroid-receptor interaction.

PHS-6040

Progress:

1 - Gynecomastia

Two etiologic subgroups of patients with gynecomastia still have an unexplained pathogenesis; pubertal and drug induced gynecomastia. Drug induced gynecomastia can have several theoretical causes: 1 - the drug acts as an estrogen; 2- the drug acts as an antiandrogen; 3 - the drug causes an increased production of estrogen from the testes; 4 - the drug causes a decreased secretion of androgen from the testis.

Over the past several years, examples of drugs acting as estrogens and anti-androgens have been documented, (i.e., digitoxin, and spironolactone). In the last year an example of each of the latter two pathogenetic mechanisms has been identified. Mysoline, a phenobarbital like anticonvulsant, has been shown to increase the metabolic clearance rate of testosterone. Plasma testosterone levels are then kept in the normal range by an increase in LH secretion. The increased LH secretion, however increases plasma estradiol concentrations, presumably because of increased testicular aromatase activity with consequent increased testicular estradiol secretion.

The second mechanism, decrease testicular testosterone secretion has been found to be a consequence of o,p'-DDD therapy for Cushing's disease or adrenal carcinoma. These patients have low plasma free testosterone levels, increased plasma LH levels and, as a result, increased plasma estradiol concentrations.

2 - Hirsutism:

A long standing hypothesis advanced to explain the occurrance of hirsutism in women with normal plasma testosterone concentrations is that they may have an increased end organ sensitivity to androgen. This hypothesis has been examined in a group of hirsute women with plasma testosterone levels in the normal range by measuring androgen receptor concentrations in fibroblast cultures from these patients. No differences were found between these women and a non-hirsute control group.

Significance:

1 - Gynecomastia - the mechanism outlined for both mysoline and o,p'-DDD has important clinical implications in that both are theoretically amenable to testosterone treatment. Such therapy would be expected to reduce LH secretion and, hence, interstitial aromatization with a resultant decrease in estradiol secretion.

2 - Hirsutism

These studies suggest, but do not prove, that increased sensitivity to androgen at the level of the target tissue does not contribute to hirsutism in most patients having this complaint in association with normal plasma testosterone values.

Proposed Course:

1 - Gynecomastia - a) studies directed toward elucidation of the pathogenesis of pubertal gynecomastia will be initiated. b) the ability of fibroblasts and lipocytes to aromatize androstenedione and testosterone will be examined. Fibroblast aromatization will be compared in normal subjects and patients with gynecomastia. Lipocyte aromatization will be compared between breast specimens with and without microscopically apparent ductular hypertrophy.

2 - Hirsutism - The efficacy of topically applied anti-androgens for the treatment of idiopathic hirsutism will be explored.

Publications:

1. Winters, S.J., and D.L. Loriaux: Effect of Chronic Castration on Testosterone and Estrogen Binding to Mouse Kidney. J. Steroid Biochem., 13: 781, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00161-05 DEB
(previously RRB)

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Role of Sex Steroids in Regulation of FSH and LH Levels in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard J. Sherins, M.D., Senior Investigator, DEB, NICHD, NIH

Other: D. Lynn Loriaux, M.D., Ph.D., Chief, DEB, NICHD, NIH
Bruce C. Nisula, M.D., Senior Investigator, DEB, NICHD, NIH

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION ~~Developmental Endocrinology Branch~~

INSTITUTE AND LOCATION

~~NICHD, NIH, Bethesda, Maryland 20205~~

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of studies in the rat have been utilized to ascertain the extent to which testosterone and estradiol can regulate the secretion of FSH and LH in the absence of the testes. The data demonstrate that 1) testosterone alone at physiologic doses can maintain both gonadotropins within the normal range, 2) that estradiol alone preferentially suppresses LH, but not FSH concentrations and 3) that a selective increase in plasma FSH is produced when there is reduced androgen production in association with increased estradiol production. Similar changes are seen in the pituitary content of FSH and LH suggesting that these altered sex steroid levels effect gonadotropin synthesis, storage and release. These data provide an alternate explanation for the inhibin hypothesis which suggests that there is a specific non sex steroid inhibitor of FSH secretion arising from the seminiferous tubules.

PHS-6040
(Rev. 10-76)

Z01 HD 00161-05 DEB
(previously RRB)

Objectives:

A considerable body of data exists suggesting that androgens regulate pituitary LH secretion in men by negative feedback inhibition. Estradiol, however, also significantly suppresses LH secretion. Since plasma estradiol in men is derived primarily from testosterone aromatized in peripheral tissues, any study of the role of testosterone per se on gonadotropin secretion measures the effect of both testosterone and estradiol. Accordingly, a series of studies have been developed to compare the relative contributions of testosterone and estradiol in the regulation of FSH and LH concentrations in normal men and male rats.

Methods Employed:

Testosterone (T) and Estradiol (E) are administered to castrate adult male rats via silastic capsules. Plasma levels of T, E, and FSH and LH are measured by radioimmunoassay from samples obtained during one month of study and correlated with changes in hypothalamic content of GnRH and pituitary content of FSH and LH.

Major Findings:

When T alone is given at a dose which maintains mid normal plasma T levels and normal prostate size in the castrate rat, plasma FSH and LH are both normal, indicating that testicular factors other than sex steroids are not necessary for maintaining FSH and LH levels within the normal range in the rat.

By contrast, when E is given alone at a dose which increases plasma E levels slightly above normal, plasma LH concentrations are suppressed, while FSH levels remain unexpectedly elevated into the castrate range. The data indicate that androgen (not estrogen) is critical in maintaining tonic suppression of FSH secretion.

When T and E are administered together at doses which produce subphysiologic levels of plasma T, a selective increase in plasma FSH is maintained similar to that seen in male animals with severe germ cell depletion. A similar change in ratio of pituitary FSH and LH is also produced at a time when GnRH content of the hypothalamus is within normal limits, suggesting that altered ratios of testosterone and estradiol primarily effect synthesis, storage and release of pituitary gonadotropins.

Significance:

These data add considerable insight into the mechanism of control of gonadotropin secretion in the male. The results indicate that testosterone can maintain both gonadotropins within the normal range in the absence of other testicular factors and, further, that a selective increase in plasma FSH level can be induced when there is subnormal replacement of T but increased production of E. The data provide an alternate explanation for the inhibin hypothesis.

Proposed Course:

1) Further studies in the castrate rat will be pursued to establish whether T and E act at the level of the hypothalamus to alter GnRh concentrations.

2) Using in vitro cultures of rat anterior pituitary cells, the effects of T and E on synthesis of FSH and LH will be investigated.

3) Using castrate men and men with germ cell depletion, a systematic study of androgen production in relationship to estrogen production will be made to determine if the selective increase in FSH seen in men with germinal aplasia can be accounted for by a decrease in T production in association with increased E production.

4) In the human, testosterone is transported in the circulation bound to TeBg (a binding protein). T reduces while E increases TeBg concentrations. Thus, subtle changes in T/E ratio can markedly alter T transport. Accordingly, TeBg binding will be assessed in men with reproductive disorders to provide a more quantitate estimate of changes in testicular function.

It is our intention to show that a subtle decrease in Leydig cell function in men with germ cell depletion produces decrease T production and increased E production leading to a selective increase in plasma FSH concentration similar to data obtained from our studies in the rat. In the human, however, T binding to TeBg in plasma may obscure the subtle changes in sex steroid production.

Publications:

Winters, S.J., Janick, J.J., Loriaux, D.L. and Sherins, R.J.: Studies on the Role of Sex Steroids in the Feedback Control of Gonadotropin Concentrati in Men, II: Use of the Estrogen Antagonist Clomiphene Citrate. J. Clin. Endocrinol., 48: 222-227, 1979.

Marynick, S.P., Loriaux, D.L., Sherins, R.J., Pita, J.C. and Lipsett, M.B.: Evidence that Testosterone Acts as Both an Androgen and an Estrogen in Suppressing Pituitary Gonadotropin Secretion in Men. J. Clin. Endocrinol., in press.

Winters, S.J., Sherins, R.J., and Loriaux, D.L.: Studies on the Role of Sex Steroids in the Feedback REgulation of gonadotropin Concentration in Men III: Androgen Resistance in Primary Gonadal Failure. J. Clin. Endocrinol., 48: 553-558, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00163-05 DEB (previously RRB)

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Clinical Studies of Male Reproductive Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard J.. Sherins, M.D., Senior Investigator, DEB, NICHD, NIH

Other: T. Mann - Visiting Scientist, DEB, NICHD
C. Gagnon - Visiting Scientist, DEB, NICHD
J.L. Ziegler - Senior Investigator, NCI, NIH
N. Javadpour - Senior Investigator, NCI, NIH

COOPERATING UNITS (if any)

C.W. Bardin, M.D., Director, Population Council Rockefeller University, New York

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A variety of studies of clinical disorders of male reproduction are in progress. These include 1) studies of the hormonal regulation of spermatogenesis in hypogonadotropic men, 2) analysis of biochemical parameters of sperm metabolism in men with non-motile sperm, 3) longitudinal evaluation of the adverse effects of cytotoxic drugs on testicular function in men receiving chemotherapy for malignant disorders, and 4) the evaluation of treatment of men with idiopathic infertility.

PHS-6040

Z01 HD 00163-05 DEB
(previously RRB)

Objectives:

The primary objective of the project is to provide a rational basis for the diagnosis and treatment of the disorders of male infertility.

Methods Employed:

The evaluation of patients with infertility and/or hypogonadism begins with a thorough examination in our outpatient clinic. Subsequent admission to our metabolic ward is used when more detailed study is required. About 100 new patients are studied annually. A variety of techniques are employed which include: measurement of hormones (FSH, LH, testosterone, estradiol, etc.) in biological fluids, semen analysis, testicular biopsy, chromosome analysis, electron microscopy of sperm and/or testis, and selected enzyme activity measurements in sperm.

Major Findings:

Studies of semen from infertile men with non-motile sperm (necrospermia) show 1) that such cells fail to metabolize fructose, the normal primary energy source in sperm, and 2) have increased specific activity of an endogenous lipid peroxidase, capable of producing peroxides of sperm phospholipid. These peroxides promptly and irreversibly immobilize spermatozoa.

Human sperm have recently been shown also to contain protein carboxyl methylase, an enzyme required for chemotaxis by mammalian monocytes and bacteria. This enzyme acts upon a specific protein methyl acceptor and inactivation of the enzyme leads to failure of characteristic unidirectional motile progression. A deficiency of protein carboxyl methylase activity has been identified in some men with immotile sperm.

Significance:

These observations suggest that there may be a biochemical marker for certain of the male infertility states in which sperm motility is altered.

Proposed Course:

1) Establish a laboratory designed to provide hormone to hypogonadotropic subjects via an implantable programmable infusion pump. Such a highly sophisticated computer programmable delivery system is now available for phase I testing, in collaboration with the Applied Physics Laboratory, Johns Hopkins University, Baltimore, Maryland. This laboratory will be established to assess the hormonal regulation of spermatogenesis, but as a core laboratory will also be available to other NICHD investigators interested in utilizing the principle of continuous infusion systems to study and/or treat other endocrine disorders.

2) Pursue studies of human sperm biochemistry and attempt to identify correlations between the parameters of fructolysis, caffeine acivation of motility, the specific activity of protein carboxyl methylase and lipid peroxidase among men with necrospermia as well as infertile men with less severe disorders of sperm motility. We will include electron microscopic evaluation of the sperm in view of recent findings in other laboratories that the sperm of some necrospermic patients show ultrastructural abnormalities (i.e., absence of the dynein arms of axial filaments, known to contain ATPase).

Most would now agree that semen analysis is often an inadequate and insensitive tool in judging the adequacy of an ejaculate for impregnation. Accordingly, we are evaluating a spectrum of biochemical assays of human sperm function in an attempt to refine our diagnostic abilities in this regard.

3) Assess the effect of a variety of classes of cytotoxic drugs, used as chemotherapy for cancer patients, on human reproductive function. Such patients will also be utilized to assess changes in endocrine function of the testis and the subsequent alterations in regulation of FSH and LH secretion.

4) Recent evidence indicates that reduction of estradiol production from aromatization of testosterone actually enhances testosterone production because of an auto regulatory action of estradiol on T synthesis within the testis. A preliminary clinical study has shown that Teslac, an aromatase inhibitor, can increase sperm production in some men with idiopathic infertility by increasing T production. Accordingly, a double blind clinical trial of Teslac in infertile men will be established.

Publications:

Jones, R., Mann, R. and Sherins, R.J.: Peroxidative Breakdown of Phospholipids in Human Spermatozoa, Spermicidal Properties of Fatty Acid Peroxides, and Protective Action of Seminal Plasma. Fertil. Steril. 31: 531-537, 1979.

Gagnon, C., Sherins, R.J., Mann, r., Bardin, C.W., Amelar, R.D. and Dubin, L.: Deficiency of Protein Carboxyl-Methylase in Spermatozoa of Necrospermic Patients. In: Testis, Development, Structure and Function. A. Steinberger and E. Steinberged (Eds.), Raven Press, 1979, p. 491-496.

Mann, T., Jones, R. and Sherins, R.J.: Oxygen Damage, Lipid Peroxidation and Motility of Spermatozoa. In: Testis, Development, Structure and Function. A. Steinberger and E. Steinberged (Eds.), Raven Press, 1979, 497-502

Schilsky, R.L., Lewis, B.J., Sherins, R.J. and Young, R.C. Gonadal Dysfunction in Patients Receiving Chemotherapy for Cancer. Annals Int. Med. 93: 109-114, 1980.

Shamberger, R.C., Sherins, R.J. and Rosenberg, S.A. The Effects of Postoperative Adjuvant Chemotherapy and Radiotherapy on Testicular Function in Men Undergoing Treatment for Soft Tissue Sarcoma. Cancer (in press).

Mann, T., Jones, R. and Sherins, R.J. Fructolysis in Human Spermatozoa Under Normal and Pathological Conditions. J. Androl. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00180-02 DEB (previously RRB)

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Biology of Hormone Binding Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bruce C. Nisula	Sr. Investigator	DEB, NICHD
Other:	James F. Dunn	Clinical Associate	DEB, NICHD
	Michel M. Pugeat	Visiting Fellow	DEB, NICHD
	Richard Sherins	Sr. Investigator	DEB, NICHD
	D. Lynn Loriaux	Head	DEB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Summary:

The overall objective of this project is to understand the role of serum hormone binding proteins in normal physiology and in the pathophysiology of various human disease states. Novel methods with improved precision and sensitivity for the measurement of the principal human sex hormone transport proteins, testosterone-estradiol binding globulin (TeBG) and albumin, have been developed. Structure-function studies have revealed unexpectedly high affinity TeBG binding of a group of estrogen metabolites which have no intrinsic sex hormone activity, the 2-methoxyestrogens. Future directions of the project include (1) the development and characterization of an animal model in which the physiologic role of the steroid binding globulins can be investigated and (2) a detailed assessment of the nature of sex steroid transport throughout human development with particularly emphasis on puberty and its disorders.

PHS-6040

Z01 HD 00180-02 DEB
(previously RRB)

Objectives:

The purpose of this project is to investigate the role of steroid hormone binding in the control of physiologic processes and in the pathophysiology of various disease states. In addition to defining the transport of steroid hormones in normal individuals, this will require examination of steroid transport in conditions characterized by altered hormone binding. Over the past year attention has focused on the application new methodology for the characterization of steroid transport to the study of the transport of testosterone in a variety of normal and disease states.

Methods:

Total steroid hormone levels are measured by standard radioimmunoassay techniques employing separative procedures when necessary. Parameters of steroid-protein binding are studied primarily using a solid phase method developed in this laboratory which utilizes the affinity of the plant lectin concanavalin A for binding glycoproteins. In addition, equilibrium dialysis, ultrafiltration and gel chromatography may be used. Computer models are used to solve complex equilibrium problems and calculate the *in vivo* distribution of steroid hormones among their major transport proteins.

Participants in this study include normal volunteers and patients with a variety of developmental and reproductive abnormalities.

Progress:

Testosterone transport is characterized using a solid phase method developed in this laboratory. Adsorption of TeBG from serum samples onto a solid phase matrix of Con A-Sepharose permits examination of testosterone binding to TeBG both before and after the removal of the endogenous serum albumin. Appropriate Scatchard analysis of this binding allows calculation not only of the TeBG binding capacity, but also the TeBG association constant for binding testosterone and a parameter of albumin binding equivalent to the product of its affinity and capacity for binding testosterone. By applying the law of mass action, these testosterone binding parameters together with the total testosterone concentration can be used to calculate the distribution of circulating testosterone into TeBG bound, albumin bound and unbound concentrations. This methodology has been applied to the study of testosterone transport in normal men and women. These studies demonstrate that women have a significantly higher TeBG binding capacity and a lower association constant than men. There was no difference in the albumin binding parameter between men and women.

A survey of the binding of endogenous steroids to TeBG has identified the 2-methoxyestrogens as a new class of steroids which bind with high affinity to TeBG. Examination of the ability of unlabeled steroids to displace labeled testosterone from TeBG absorbed onto a solid phase

matrix of Con A-Sepharose permits determination of the relative binding activities of unlabeled steroids for binding TeBG. Systematic study of the binding of over twenty-five endogenous steroids in this manner has provided reliable values for their TeBG binding affinities. This survey also identified two compounds with high affinity binding to TeBG which were previously unrecognized--2-methoxyestrone and 2-methoxyestradiol. Detailed examination of the binding of these estrogen metabolites to TeBG revealed that 2-methoxyestradiol binds to TeBG with greater affinity than testosterone, and that both 2-methoxyestradiol and 2-methoxyestrone bind to TeBG with greater affinity than estradiol.

Significance:

The available evidence suggests that only the portion of testosterone which is not bound with high affinity to TeBG in vivo is available for uptake by target tissues. Therefore, critical evaluation of the biologic action on testosterone should include not only determination of the total testosterone concentration but also its distribution among the major transport proteins. These techniques have been developed and are currently being used in clinical investigations of a variety of disorders such as hypogonadism, gynecomastia, hirsuitsm and disorders of sexual development.

The observation that 2-methoxyestradiol binds to TeBG with greater affinity than tesotosterone has provided new insight into the structure-function of TeBG since the presence of a methoxy group in the 2 position enhances the binding of the parent estrogen to TeBG. That this high affinity binding is not shared with the androgen receptor suggests that major differences exist in the structural requirments for binding to TeBG and the androgen receptor. In this regard, the 2-methoxyestrogens represent the first examples of naturally-occuring steroids which bind with high affinity to TeBG but with low affinity to both estrogen and androgen receptors. This raises the interesting possibility that compounds with no intrinsic sex hormone activity could exert physiologically significant effects by displacing the potent endogenous sex hormones from TeBG.

Proposed Course:

Clinical investigations will continue to characterize the binding of steroid hormones in normal individuals with particular interest in the changes in hormone secretion and binding which occur during puberty. Studies into the pathogenesis of gynecomastia will be extended to include not only adults with idiopathic gynecomastia and gynecomastia secondary to other disease processes, but also boys with physiologic pubertal gynecomastia. Evaluation of men with testicular disorders will focus on the feedback control of gonadotropins with particular attention to hormone binding where discrepancies between the observed clinical state and total hormone concentrations are observed.

A second major transport protein, corticosteroid binding globulin (CBG) is also adsorbed by concanavalin A. Modification of our solid phase method to study the binding of CBG will permit extensive evaluation

of the binding of endogenous steroids to both TeBG and CGB will allow computer simulation of the transport of over 20 endogenous steroid in a variety of physiologic and pathologic conditions. In addition, a systematic survey of the binding of pharmacologic compounds to both TeBG and CBG together with an assessment of the in vivo impact of this binding on the transport of endogenous steroids is planned. In addition to providing information on the biologic availability of these compounds when given in therapeutic doses, these binding studies are expected to provide additional insight regarding the structure-function requirements of the transport protein

Effort will also be directed to the development of laboratory model to investigate the effect of TeBG binding in vivo. This will require partial purification of TeBG utilizing the unique capacity of concanavalin A to adsorb this protein. Examination of the effects of the administration of enriched preparations of TeBG to animals which do not have this protein should provide insight into the role of TeBG binding on sex hormone transport, metabolism and action.

References:

1. Nisula, B.C. and J.F. Dunn, Measurement of the Testosterone Binding Parameters for both Testosterone-Estradiol Binding Globulin and Albumin in Individual Serum Samples. Steroids 34: 771, 1979.

2. Marynick, S.P., B.C. Nisula, J.C. Pita and D. Lynn Loriaux, Persistent Pubertal Macromastia. Journal of Clinical Endocrinology and Metabolism 50: 128, 1980.

3. Dunn, J.F., G.R. Merriam, C. Eil, S. Kono, D.L. Loriaux and B.C. Nisula, Testosterone-Estradiol Binding Globulin Binds to 2-Methoxyestradiol with Greater Affinity than to Testosterone. Journal of Clinical Endocrinology and Metabolism, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00181-02 DEB (previously RRB)

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Steroid Antagonists

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other	D. Lynn Loriaux, M.D., Ph.D., Chief	DEB, NICHD, NIH
	G. Chrousos, M.D., Clinical Associate	NPMB, NICHD, NIH

COOPERATING UNITS (if any)

Marvin Karten

LAB/BRANCH

Developmental Endocrinology Branch

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INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. No antagonists have been identified for the glucocorticoids or the progestins. The objective of this project is to develop antagonists for both of these classes of steroids.

PHS-6040

Z01 HD 00181-02 DEB
(previously RRB)

Objectives:

Clinically useful antagonists have not been developed for glucocorticoids or progestins. Antagonists for both of these classes of steroids exist *in vitro*. The objectives of this project are to understand why the known *in vitro* antagonists fail to act *in vivo*, and using this knowledge, to produce effective *in vivo* antagonists.

Methods Employed:

Methods consist of glucocorticoid and progesterone radioreceptor assays, *in vivo* tests of glucocorticoid action (thymus involution, glycogen deposition, growth retardation) and progesterone action (endometrial maturation index).

Progress:

11-deoxycortisol (S) is a known glucocorticoid antagonist *in vitro*. It has not previously been shown to be an antagonist *in vivo*. We found that the reason for the apparent failure of S to act as a glucocorticoid antagonist *in vivo* is that it undergoes 11-hydroxylation by the adrenal gland, thus being converted to an agonist. This has been confirmed by showing that S retains full antagonist activity in adrenalectomized rats. Work has progressed toward developing effective *in vivo* antagonists by developing S analogues that resist 11-hydroxylation, such as $\Delta^{1,9(11)}$S. This compound acts as a glucocorticoid antagonist both *in vivo* and *in vitro*. However, this compound is not sufficiently resistant to 11-hydroxylation to have potential clinical usefulness.

Significance:

The early results of these studies suggest that it will be possible to develop effective antiglucocorticoids. These compounds, if active in man, would have great promise as a therapeutic tool and as an adjunct to studying adrenal physiology.

Proposed Course:

1) Identification of glucocorticoid antagonists. A new analog of 11-deoxycortisol is being synthesized which promises to be completely resistant to 11-hydroxylation. As soon as available this analog will be tested for antiglucocorticoid activity.

Publications:

1. Chrousos, G.P., K.M. Barnes, M.A. Sauer, D.L. Loriaux, and G.B. Cutler, Jr.: $\Delta^{1,9(11)}$-11-Deoxycortisol: An improved Glucocorticoid Antagonist. Endocrinology 107:472, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00182-02 DEB (previously RRB)

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Structure, Function, and Physiology of Glycoprotein Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Bruce Nisula	Senior Investigator	DEB	NICHD
Other:	Sania Amr	Visiting Fellow	DEB	NICHD
	Pierre Carayon	Guest Worker	DEB	NICHD

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad objective of this project is to understand the structure, function and physiology of the glycoprotein hormones: thyrotropin, luteinizing hormone, follicle stimulating hormone, and chorionic gonadotropin. Recent research developments concerning chorionic gonadotropin include the following: establishment of the renal clearance rates of the subunits of the hormone, characterization of a discrete fragment which is a unique degradation product, demonstration of an estrogen-induced decrease in the testis glycoprotein receptor number and elucidation of the interaction of chorionic gonadotropin with the human thyroid by the classical glycoprotein receptor mechanism. Further refinement of the methodology for the measurement of serum thyrotropin has provided a significant extension of the clinical utility of this test. Future work will continue to focus on improving clinical methodology for the measurement of the glycoprotein hormones and the products of their metabolism, and will give more emphasis to structure-function relationships pertaining to the thyrotropin receptor.

Z01 HD 00182-02 DEB
(previously RRB)

Objectives:

The overall objective ot this project is to understand the biology of the glycoprotein hormones and the role of glycoprotein hormone derangements in human diseases. The human glycoprotein hormone group consists of thyrotropin (hTSH), chorionic gonadotropin (hCG), luteinizing hormone (hLH), and follicle-stimulating hormone (hFSH). The research effort in the current year has been directed toward: 1) Refining the clinical methodology for the measurement of serum hTSH with the goal of advancing its clinical usefulness in the evaluation of thyroid diseases; 2) Delineating the kinetics, metabolism, renal excretion, and degradation of the hCG subunits in man; 3) Elucidating the molecular mechanisms of the thyrotropic activity intrinsic to the hCG and hLH molecules.

Methods Employed:

Glycoprotein hormones and related molecules with thyroid-stimulating, luteinizing, and follicle-stimulating activities are measured by bioassay, radioreceptor assay, and radioimmunoassay. The hormones subunits, and related secretory or degradation products are extracted, concentrated, separated or purified by various procedures including gel filtration, adsorption chromatography, or affinity chromatography.

Progress:

A highly sensitive and reliable radioimmunoassay for the measurement of hTSH in human serum has been developed. The value of former radioimmunoassays for hTSH has been limited principally because of poor sensitivity and specificity such that the reliable measurement of normal serum hTSH levels has not been possible. In contrast, our new methodology permits delineation of the entire range of values in normal subjects (0.5 - 4.5 μU/ml). Results with this assay show that patients with thyrotoxicosis of diverse etiologies (diffuse toxic goiter, toxic multinodular goiter, subacute thyroiditis, and thyrotoxicosis factitia) have serum hTSH levels suppressed well below the normal range. The procedure involves the concentration of hTSH from serum by adsorption to concanavalin-A covalently linked to 4B Sepharose, lactoperoxidase iodination of the hTSH, and disequilibrium assay conditions. Data accummulated to date clearly indicate its value as a diagnostic tool in hyperthyroidism.

We have investigated the kinetics, metabolism, renal excretion, and degradation of the hCG subunits in human subjects. The mean renal clearance rate of hCG-alpha and hCG-beta are in the 0.2-0.3 ml/min range. While the total metabolic clearance rate of hCG-alpha is about 3-fold that of hCG-beta, their renal clearance rates are similar. It was surprising to find that renal excretion accounted for less than 1% of the total disposal of the subunits. Investigation of the different molecular forms of immunoreactive hCG-beta which arise during its metabolism, revealed a single discrete degradation product of hCG-beta. Wide heterogeneity in the molecular forms of degraded subunit was not found. The data show that

a metabolic pathway exists in humans which degrades hCG-beta to a discrete carbohydrate-containing fragment which lacks the carboxyterminal peptide region immunologic determinant and which exhibits an apparent molecular weight on G100 Sephadex about 25% that of the intact hCG-beta molecule.

Progress has been made in the study of structure-function relationships among the glycoprotein hormones. Our earlier work with rodent species had suggested the concept that the selectivity of the interaction between hormone and target cell was relative, not complete. Thus, interstitial cell stimulating hormones, like hCG and hLH, exhibit a weak intrinsic thyrotropic activity in rodent bioassays. To determine if this concept could be extended to the human species, studies of the interaction of hCG and hLH with human thyroid membranes were carried out. Indeed, both hCG and hLH bind to and stimulate the adenylate cyclase activity of human thyroid membranes. An unexpected result was the finding that hLH is about 65-fold more potent than hCG. As these 2 molecules are otherwise functionally quite similar, it is appealing to attribute this functional difference to the most obvious structural difference, the extra 30 amino acids at the carboxy-terminus of hCG-beta. These observations provide a molecular basis for the thyrotropic activity of hCG and hLH, give rise to a hypothetical role for the extra carboxy-terminal region on hCG, and point to key structural differences between thyroid and testis glycoprotein receptors.

A clinical study aimed at exploring thyrotropic factors by porcine thyroid radioreceptor assay in hyperthyroid patients with choriocarcinoma has been completed. There was no evidence of a thyrotropic factor other than hCG in the urine of these patients. These studies with porcine thyroid membranes, like those with human thyroid membranes, clearly point to an interaction of hCG with the TSH receptors.

A study of the effect of estradiol on the testis has shown that hCG receptor number can be modulated by the steroidal milieu. In rats, treatment with estradiol produced a 30% decrease in Leydig cell hCG binding. Testosterone biosynthesis was inhibited with an apparent reduction in 17-20 desmolase activity.

Our studies of the kinetics and metabolism of the glycoprotein hormones have led to analogous studies of another glycoprocein, dopamine-beta-hydroxylase. The levels of this glycoprotein in rats were initially felt to be an ideal indicator of synthetic activity; however, physiologic studies failed to bear out this concept. Recent work on the catabolism and kinetics of this glycoprotein imply a major role of its catabolic pathways. Further studies are expected to give greater perspective to the relative roles of sympathetic activity and catabolism in modulating serum levels of the enzyme.

Significance:

These studies have extended our knowledge of various clinical, biochemical, and pathophysiological aspects of the glycoprotein hormone area. Our refined radioimmunoassay for serum hTSH can be expected to extend the clinical utility of hTSH measurements substantially. It has been believed for some time that serum hTSH levels are suppressed below the normal range in patients with thyrotoxicosis, but the data supporting this concept have been few and there has been no clinical application of this putative physiological principle. Thus, our initial observations with the new methodology, contribute to the establishment of this principle, and, in so doing, they point the way to multiple clinical applications which have been heretofore not feasible. The experiments showing that hCG binds to and stimulates the adenylate cyclase activity of human thyroid tissue indicate that a classical glycoprotein receptor interaction is the biochemical mechanism for the expression of the thyrotropic activity of hCG and hLH and that these molecules are indeed hTSH agonists. This result gives important support to the hypothesis that hCG is the thyrotropic factor that mediates the hyperthyroidism which occurs in patients with hCG-secreting neoplasms. Analysis of structure-function relationships has led to the novel and intriguing idea that the unique carboxy-terminal peptide region of hCG actually serves to attenuate the thyrotopic activity of the molecule relative to that of hLH. The investigations of hCG subunit kinetics and metabolism have provided the first characterization of this area in human subjects and the first demonstration that a discrete degradation product of hCG or its subunits arises during metabolism. Another useful observation to come from this work is that urinary excretion as unaltered subunit accounts for a very small fraction of the total metabolic fate of the hCG subunits in man. The clinically relevant consequence is that monitoring the hCG subunits as tumor markers is performed more sensitively and efficiently in serum than in urine.

Proposed Course:

Future studies will continue to emphasize the development of more accurate, sensitive, and convenient techniques for the measurement of glycopeptide hormones, their subunits, and related degradation products in human biological specimens. Specifically, refined assays for urinary hCG, hLH, and asialo-hCG will be developed and relevant clinical situations investigated. With detailed knowledge of the kinetics and disposal of the hCG subunits in hand, we can now explore the metabolism of purified hCG in human subjects with valuable insights. We will assess whether the subunits are intermediates in the metabolism of hCG and the extent to which alternative degradation products are apparent. Clinical studies are being conducted to define new clinical applications of our ultrasensitive TSH assay and to understand the roles of TSH and hCG in trophoblastic thyrotoxicosis. Structure-function relationships at the human TSH receptor will be analyzed in greater detail. The hCG molecule will be enzymatically digested with the aim of modifying the carboxyterminal region and determining whether the alteration in molecular structure results in enhanced hTSH agonist activity as would be predicted from our studies with hLH.

Publications:

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6. Carayon, P., G. Lefort, and B. Nisula, Interaction of Human Chorionic Gonadotropin and Human Luteinizing Hormone with Human Thyroid Membranes, Endocrinology 106:1907, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00183-02 DEB
(previously RRB)

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Catechol Estrogens: physiological effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D.L. Loriaux, M.D., Ph.D.	DEB, NICHD
Other	G. Merriam, M.D., Clinical Associate	DEB, NICHD
	S. Kono, MD., Visiting Scientist	DEB, NICHD
	M.B. Lipsett, M.D.	Director, Clinical Cen
	D.D. Brandon	DEB, NICHD

COOPERATING UNITS (if any)

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NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The catechol estrogens, 2 or 4 hydroxy derivatives of estrone and estradiol, represent major metabolites of the naturally occurring estrogens. Little is known of the origin, disposition, and metabolic effects of these compounds. The objective of this project is to address these questions.

PHS-6040
(Rev. 10-76)

Objectives:

Catechol estrogens have been identified in urine, and have been shown to be metabolic products of both estrone and estradiol. The biological effects of these compounds remains a controversial issue. The objectives of this project are to define the metabolic origin of the catechol estrogens, and to delineate their biological effects in man, using gonadotropin suppression as a response parameter, and in the rat, using uterine weight as an end point.

Progress:

1 - Biologic Action & Potency of the Catechol Estrogens

Both 2-OH estrone and 2-OH estradiol have been shown to interact with the rat uterine cytosol receptor with about 5% the affinity of estradiol.

2-OH estrone has been shown to be an estrogen agonist in man. Intravenous infusions of between 2 & 6 milligrams daily for 4 days are needed to demonstrate the suppresive effect on gonadotropin secretion. 2-OH estradiol is also an estrogen agonist in man. The 2-OH estradiol effect is demonstrable at doses of 400 - 800 µg a day for 4 days.

In the rat, no estrogenic or antiestrogenic activity for 2-OH estrone could be found even at doses more than 500 times the dose that would be predicted to be effective based on receptor affinity studies.

2. Metabolism of the catechol estrogens.

The potency studies outlined above uncovered a major discrepancy between receptor affinity and minimal effective dose for biologic action. The most likely explanation for this discrepancy is pharmacokinetic. We initiated our studies of the pharmacokinetics of the catechol estrogens in this area by examining the metabolic clearance rates of 2-OH-estrone and 2-OH estradiol in man and rat.

The MCR of 2 OH estrone in man ranges between 20,000 & 40,000 L/day. That of 2-OH estradiol is about half this. These clearance rates are 10-20 times greater than the clearance of any other known naturally occurring steroid. Because liver blood flow is rarely greater than 2000 L/day, and cardiac output is on the order of 7,000 L/day, these data suggest that a large portion of the clearance must be accomplished by the blood itself. Subsequent studies have confirmed that 2-OH-estrone and 2-OH estradiol are rapidly metabolized by erythrocyte catechol-O-methyl transferase, and that the reaction rate can account for the rapid clearance of the catechol estrogens from blood.

These findings explain why such large doses of the catechol estrogens are needed to elicit biological effects.

Because of the need for large doses, of catechol estrogens small amounts of an active contaminant could potentially account for all or part of the observed effects. This probably is the explanation for the controversial actions attributed to the catechol estogens in the literature. Our findings of estrogen agonism are compatible with the measured MCR's, estrogen receptor affinities, and cannot be attributed to contaminants in our purified reagents.

3 - Plasma concentrations of the catechol estrogens.

Previous work with this class of steroids using radioimmunoassays suggested that plasma 2-OH estrone was present in human blood in concentrations ranging between 20-50 pg/ml for men up to several hundred pg/ml in pregnant women.

These data for non-pregnant adults are not compatible with the enormous MCR's that we have found for these compounds. Hence, we have developed a modified 2-OH estrogen radioimmunoassay in order to reevaluate the plasma concentration of these steroids. We find that plasma 2-OH estrone & 2-OH estradiol do not circulate in measurable quantities in non-pregnant adults (<10 pg/ml) and range up to 200 pg/ml in pregnant women. This is a considerable revision downward of the previously accepted plasma concentrations for these steroids, but are the only values compatible with the known metabolic clearance of these steroids and the production rates of their known precursors.

Projected Course:

The mechanism of action of the catechol estrogens in the central nervous system remains unclear. Two hypotheses prevail. The first proposes that these steroids act exclusively via catecholamine receptors or by influencing the metabolism of endogenous CNS catechol amines. The second proposes that all of the actions of the catechol estrogens can be accounted for by their interaction with the estrogen cytosol receptor. We propose to test these two hypotheses by examining the action of the catechol estrogens in systems wherein one or the others of these receptor systems has been blocked. Initial studies will use the rat as the experimental animal.

Publications:

1. G.R. Merriam. Catechol estrogens: a brief review. J. Am. Med. Women's Assoc. 34:363-370, 1979.

2. G.R. Merriam, N.J. MacLusky, M.K. Picard, and F. Naftolin. Comparative properties of the catechol estrogens. Methylation by COMT and binding to cytosol estrogen receptors. Steroids, July 1980 (in press).

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6. S. Kono, D.D. Brandon, G.R. Merriam, D.L. Loriaux, and M.B. Lipsett. Low plasma levels of 2-hydroxyestrone are consistent with its rapid metabolic clearance. Steroids, in press, 1980.

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